

BEHAVIORAL AND SENSORY ASSESSMENT OF WILE
WITH AN ELECTRONIC NOSE

By

FRANK KOBEL

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To my parents, Zenna and Moses Karel, for all their love, encouragement, support and for the wonderful opportunity they gave me throughout my life to obtain the best education possible

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**MICROBIAL AND SENSORY ASSESSMENT OF MILK
WITH AN ELECTRONIC NOSE**

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Fayez Kord

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Important psychotropic microorganisms in raw milk are Gram-negative rods with *Pseudomonas* spp. comprising 63-to 100% of the genera. Some Gram-positive bacteria are also present, with *Bacillus* being the most important genus. Spoilage bacteria found in raw milk produce heat-stable lipases and proteases that are not destroyed by pasteurization. In general, microbial counts in excess of 1×10^6 cfu/ml are enough to produce defects in milk.

Several off-flavors in milk, such as bitter, putrid, urethane, rancid, and sour, have been associated with psychotropic microorganisms. Traditional microbial evaluation of milk is time consuming. Faster methods are desirable. The electronic nose is a promising technology that can be used as a fast screening tool. It reduces objectivity of flavor evaluation, requires minimal sample preparation, generates reproducible and reliable

results, is easy to operate, and results can be obtained quickly. The objectives of this study were to test an electronic nose for the assessment of milk associated with *Pseudomonas fluorescens* and/or bacterial coagulation, to correlate microbial loads and sensory results with electronic nose readings, and to attempt to predict shelf life based on microbial loads of milk samples in an accelerated study.

PennState® whole, reduced-fat, and fat-free milk were used. Sterile milk samples were inoculated with *P. fluorescens* and/or *B. coagulans*, stored at 1 °C, 5 °C, and 12 °C, and evaluated at days 0, 1, 3, 5, 7, and 10 using an electronic nose. Counts for *P. fluorescens* were performed using serials plate count (MC Platelet). Those for *B. coagulans* were performed using nutrient agar plates. The odor of milk samples was evaluated by a 10-member untrained sensory panel. Electronic nose readings, microbial counts, and sensory data were analyzed using discriminant function analysis. The electronic nose discriminated differences in odor due to microbial load, storage temperature, and sensory data. This research demonstrated the potential use of electronic nose to detect odor differences in milk due to microbial loads. Electronic nose readings can be correlated with sensory panel perception. This may lead to a new rapid method for determining sensory evaluation and microbial loads of milk.

CHAPTER I INTRODUCTION

Milk is a good medium for growth of pathogens and spoilage organisms. Raw milk contains varying numbers of microorganisms, depending on the care employed in milking, cleaning, and handling of milk vessels. Raw milk held at refrigeration temperatures for several days shows the presence of several bacteria of the following genera: *Pseudomonas*, *Listeriacoccus*, *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Microbacterium*, *Moraxella*, *Propionibacterium*, *Acidithiobacillus*, *Proteus*, *Brucella*, and some others. These title to grow at low temperatures and so increase in numbers (Brenner and McKee, 1960).

Defects in milk can arise from four sources: the growth of psychrotrophic organisms prior to pasteurization, the activity of thermotolerant enzymes, the growth of thermotolerant psychrotrophic organisms, and post-pasteurization contamination. Psychrotrophic organisms, although mostly not thermotolerant, are important because many produce extracellular thermotolerant proteolytic and lipolytic enzymes which can survive pasteurization and even ultra high temperature (UHT) processing (Rane and Gilmer, 1985). These extracellular enzymes hydrolyze milk proteins and lipids and cause off-flavors in UHT milk. In general, psychrotrophic counts in excess of 1×10^2 colony forming units (cfu)/ml are reported to produce defects in quality (Krause, 1982).

The dairy industry needs a simple, rapid, sensitive, reliable, and economical method for assessing psychrotrophic organism population in raw and pasteurized milk. A fast, non-complex, and inexpensive method for reliable detection of psychrotrophic organisms will be a valuable quality control tool for the dairy industry.

Each type of bacteria has a "signature" of volatile products that form a unique odor. The method of using the bacteria can be to "smell" the bacterial metabolism by the use of sensor arrays, also known as electronic noses. Due to its sensitivity, the electronic nose has a great potential in microbiological analysis. Six types of bacteria (*Enterobacter aerogenes*, *Proteus*, *Escherichia coli*, *Acetivibrio fragilis*, *Clostridium* and *Pseudomonas aeruginosa*) were discriminated by using a 4-element metal oxide sensor array. *Escherichia coli* and *Staphylococcus aureus* were also discriminated (Gierke and Crona, 1996). *Pseudomonas* species, which produce different kinds of volatile metabolites, were separated by an electronic nose (Silva et al., 1995). Electronic noses have many applications in the food industry, such as monitoring the deterioration of shrimp (Kawaguchi and Ishikawa, 1999a), tuna (Niemenen, 1998), and ground meat (Wijnhout et al., 1997). Therefore, electronic noses have the potential to detect and discriminate microorganisms based on volatile bacterial metabolism.

CHAPTER 2 LITERATURE REVIEW

Milk Quality Assessment

Milk is an important element of a balanced diet (Silva, 1998). The major nutritional components of milk and their normal concentrations are 87.3% water, 4.0% lactose, 4.2% fat, 3.35% protein, and 0.68% minerals. It is a good source of B vitamins and minerals such as iron, copper, cobalt, and molybdenum (Frank, 1997). Indeed, milk is not only an excellent food for humans, it is also an ideal medium for the growth of microorganisms (Silva, 1998).

Carbon sources in milk are lactose, protein, and fat. Many microorganisms cannot utilize lactose, and therefore proteolysis or lipolysis must occur for them to obtain carbon and energy. However, some spoilage microorganisms may utilize lactose to produce lactic acid. The amount of lactose present in milk is enough to support extensive microbial growth. The cream in milk can be used by many microorganisms, but the amount is not sufficient to support significant growth. There is sufficient glucose in milk to initiate the growth of some microorganisms (Frank, 1997).

Two types of proteins, casein and whey proteins, are present in milk. Caseins are found in the form of highly hydrated micelles and are readily susceptible to microbial

proteolysis. Whey proteins (β -lactoglobulin, α -lactalbumin, serum albumin, and immunoglobulin) remain soluble in the milk after casein precipitation. In contrast to casein, whey proteins are less susceptible to microbial proteolysis (Frank, 1997).

Milk has significant fat content, but only few spoilage microorganisms utilize the fat as a carbon or energy source. The fat is in the form of globules surrounded by a protective membrane composed of glycoproteins, lipoproteins, and phospholipids. Unless the globule membrane is physically damaged or enzymatically degraded, it cannot be utilized by microorganisms (Alamoudi et al., 1997).

Milk Spoilage

From a milk spoilage perspective, psychrotrophic microorganisms are the single most important group. They are defined as bacteria that grow at 7°C or below, regardless of their optimal growth temperature. These organisms have remained an important part of the microbial flora of raw milk, since the introduction of bulk refrigerated storage. Growth of psychrotrophic microorganisms in raw milk can lead to quality and flavor defects in products made from that milk because of the residual activity of degradative heat-stable enzymes produced by these microorganisms (Brennery and McKeehan, 1994; Frank, 1997).

Milk, produced at ambient temperatures without refrigeration, must be cooled to about 3-5°C in the farm. The initial microflora, the numbers, and types of microorganisms in milk immediately after production, reflect environmental contamination during production. The cooling after production inhibits general growth of bacteria. The temperature is

what raw milk has been stored, the duration of milk storage and the storage temperature control have can affect the numbers and types of microorganisms present in raw milk (Bransby and McKinnon, 1990). Once milk leaves the farm, active refrigeration stops, and the temperature of the milk rises by at least 1°C per day. Any temperature rise will reduce the growth of psychrotrophic microorganisms (John and Hobbins, 1994).

The psychrotrophic microorganisms in milk produce a range of extracellular enzymes which can readily degrade milk constituents. These microorganisms have either lipolytic or proteolytic or combined degradation ability. Even though psychrotrophic organisms can be killed by pasteurisation or ultra-high temperature (UHT) processes, their enzymes cannot be inactivated (Bransby and McKinnon, 1990). Growth of psychrotrophic microorganisms in milk can lead to spoilage because of the heat-stable degradative enzymes of these organisms. Consumers can detect these quality and flavor defects.

There are three main sources of microbial contamination of milk during production: from within the udder, from the exterior of the teats and udder, and from the milking and storage equipment (Bransby and McKinnon, 1990). Soil, water, animals, and plant materials are sources of psychrotrophic microorganisms found in milk. The exterior of the teats and udder can harbor high levels of psychrotrophic bacteria, even after milking and washing.

Water used on the dairy farm usually contains low populations of psychrotrophs. Its use to clean and rinse milking equipment provides a direct contamination route milk,

Psychrotrophs isolated from water are often very active producers of extracellular enzymes, and grow rapidly at low temperatures (Creswell, 1982).

Plant materials used for animal feed have been found to contain 10⁷ psychrotrophic organisms/g (Chikuma, 1986). Milking and storage equipment are also major sources of psychrotrophic contamination of raw milk. Proper cleaning and sanitizing procedures can reduce contamination from such equipment. Good handling practices reduce contamination and prevent high microbial counts, and the possible presence of undesirable bacterial enzymes (Jensen and McKinnon, 1990).

Ram 2(S):

The main psychrotrophic microflora present in raw milk are aerobic Gram-negative rods with *Pseudomonas* spp. (*P. fluorescens*, *P. putida*, *P. fragi*, *P. aeruginosa*) forming 60 to 70% of the genera. *P. fluorescens putrefaciens* (Jensen and McKinnon, 1990; Creswell et al., 1989; Rasmussen et al., 1990). *Acidimicrobacter*, *Acetivibacter*, *Aeromonas*, *Alcaligenes*, *Citrobacterium*, *Flavobacterium*, and *Yersinia* form the other genera present in milk (Jensen and McKinnon, 1990; Minszberg, 1979).

Some Gram-positive bacteria are also present in raw milk, but their numbers are much smaller than those of Gram-negative bacteria. *Acetivibacter*, *Bacillus*, *Chromobacter*, *Corynebacterium*, *Enterobacter*, *Lactera*, *Microbacterium*, *Mycobacterium*, *Spirillum*, *Spherotheca*, and *Streptococcus* are isolated from milk, and *Acetivibacter* and *Bacillus* are the most common (Jensen and McKinnon, 1990).

Psychrotrophs cause an "acidic" flavor in milk, and there is a significant correlation between total psychrotrophic counts and storage temperature of raw milk at

both 2°C and 4°C. A decrease in storage temperature from 4°C to 2°C leads to a 33% increase in storage life of raw milk. This was concluded by measuring the time taken for the microbial count to reach 1×10^6 cfu/ml (Griffiths et al., 1988a). Griffiths et al. (1988b) also investigated the effect of storage of raw milk at 2°C and 4°C on the subsequent quality of pasteurised and UHT milk. Good quality pasteurised milk could be produced from raw milk stored at 2°C for up to 5 days, but the quality could be achieved with the raw milk samples which had been stored at 4°C for only 3 days. The quality of UHT milk was much higher in products manufactured from raw milk stored at 2°C for 4 days than in those stored at 4°C for 4 days (Griffiths et al., 1988b).

Aerobic, spore-forming bacteria do have frequent changes in minimum growth temperatures. Goodfellow and Hooper (1974) stated that isolated *Bacillus spp.* subsequently lost their ability to grow at $\geq 2^\circ\text{C}$ or below when they were stored at 20°C. However, mesophilic strains of *Bacillus spp.* have been adapted to grow at low temperatures by repeated transfers to media at colder temperatures over a long period of time.

Even though the majority of spore-forming organisms previously reported in milk were identified as *Bacillus spp.*, anaerobic sporeformers are also present in milk. *Mycobacterium* and *Moraxella* spp. are derived almost exclusively from milking equipment, and thermotolerant counts in the milk sometimes exceed 10^6 cfu/ml. Most of the thermotolerant organisms do not multiply appreciably in raw milk even at ambient temperatures, however, a high thermotolerant count in milk is reliable evidence of cross contamination from milking equipment (Dransky and McKeown, 1980).

The presence of coliforms and *E. coli* in raw milk is evidence partly of direct faecal contamination, and partly inadequate cleaning of milking equipment since coliforms can easily build up in teats, milky residues in milking equipment, and because the major source of contamination. Coliform counts higher than 100/plaid are considered as evidence of unsatisfactory production hygiene. Unsanitary coliform counts may also cause high coliform counts (Bourley and McKinnon, 1986).

All important spoilage bacteria found in raw milk have the potential to produce extracellular degrading enzymes, heat resistant spores, and proteases, regardless of growth conditions. When they are produced, they are not destroyed by sample heat treatment. These enzymes can play a role in the quality degradation of milk.

The fluorescent pseudomonads and bacteria in the genus *Alcaligenes* show the highest incidence of degradative action. *P. fluorescens* and *P. fragi* are the major microorganisms causing lipolytic spoilage. It was found that *P. fragi* strains were more lipolytic than those of *P. fluorescens*, and grew better at 1°C (Shelley et al., 1987). Quantitative arguments show little proteolysis and only limited lipolytic activity. The strains of *Stenotrophomonas* *Bacillus* spp. are frequently only proteolytic, and their proteolysis and spores are less heat-stable than those from *Pseudomonas* spp. (Kierkegaard et al., 1986). *Pseudomonas fluorescens* and *Bacillus cereus* also produce extracellular phospholipase C which can degrade the milk fat globule membrane leading to product defects. The phospholipase C from *Bacillus cereus* produces "fatty" cream, and inhibits rennet activity in pasteurized milk.

The production of protease and lipase by psychrotrophic bacteria is not observed until all the cells are in the late exponential or stationary phase of their growth cycle. Such a stage with most cultures of dairy origin is reached when the cell population reaches 10^7 cells/ml. This concentration of microorganisms corresponds with the onset of noticeable spoilage in raw milk (Moser et al., 1994).

A reduction in oxygen tension of the growth medium immediately promotes protease synthesis by a strain of *Pseudomonas fluorescens* (Brown and Gilmore, 1983). Griffiths and Phillips (1984) reported that protease synthesis by psychrotrophs in milk could be inhibited by maintaining high oxygen concentrations. However, other researchers stated that this effect may be strain specific, since they found that some bacteria continue to secrete both protease and lipase when the oxygen tension in the growth medium is high (Ding and Fox, 1985; Fox and Sengprad, 1985).

The effect of growth temperature on the production of proteases and lipases by psychrotrophic bacteria may also be strain specific. Growth temperature of 1°C depressed the synthesis of both protease and lipase by several pseudomonads (Griffiths et al., 1984a). This result shows that deep cooling of milk to 1°C extends its shelf life.

Estimated shelf

Pasteurization ensures the microbiological safety of milk as well as a reasonably acceptable shelf life (Moser, 1995). However, increasing the pasteurization temperature does not necessarily result in an increased shelf life because of the activation of spores at the higher temperatures (Phillips and Griffiths, 1990).

In pasteurization, milk is heated to and retained at a temperature not less than 62 °C, and not more than 65 °C for at least 30 min, then immediately cooled to below 10°C or below 6°C. Alternatively, the milk may be retained at a temperature of not less than 71 °C for at least 15 sec, then immediately cooled to less than 10°C. This process is called the high-temperature short-time method (HTST). Another method, super-pasteurization, is used in many places today. Milk is heated to and retained at a temperature of 82°C for 3 sec, then immediately cooled to 10°C (Brennery and McKee, 1996; Vais, 1987).

Pasteurization of milk was designed as a method of heating milk to kill *Apicomplexan* infections: the organism that causes tuberculosis is human. However, *Coccidia* *thermophilus*, the organism that causes Q fever, is found to be more resistant to heat than *bc* infections. Now the target organism for pasteurization is *C. burnetii*. The effectiveness of pasteurization is measured by measurement of the residual level of alkaline phosphatase (Vais, 1987).

Microbial contamination of pasteurized milk occurs through post-pasteurization contamination by psychrotrophs and through survival of thermotolerant psychrotrophic organisms during pasteurization. Many of the Gram-negative organisms do not survive pasteurization; in fact, only one species of Gram-negative bacterium, *Abundantia* *thermophilus*, can survive since it is generally considered as thermotolerant (Blair, 1990).

Spoilage-forming bacteria of the genus *Bacillus* form the most important group of microorganisms capable of surviving pasteurization, and growing in milk. The occurrence of psychrotrophic, spore-forming bacteria in pasteurized milk was first reported in 1961

The quality loss and unsatisfactory of pasteurized milk (stored at 4°C for four weeks) was observed due to the outgrowth of *Bacillus coagulans* (Kinschopf and Harper, 1934). They also isolated *Bacillus coagulans* from pasteurized milk that had been stored at 2°C for 13-to 17 days. They stated that the generation time for *B. coagulans* was 24 to 36 hrs under these refrigeration conditions.

A seasonal variation in the numbers of the genus *Bacillus* in raw milk is observed, and these bacteria predominate in the period of June to October. *B. licheniformis* is the most common species (Shewley and McCrann, 1939). During this period there is a peak in the number of spores (billion) in milk with lower count, or milk the defect known as "dry" cream (Mott, 1939). The only pathogen of the genus *Bacillus* is *B. cereus*, and it can survive, and grow at refrigeration temperature (Shewley and McCrann, 1939).

Researcher in the US reported that psychrotrophic sporeformers were isolated from 28-55% of badly pasteurized milk (Mansel et al., 1931). Others observed heat activation of *Bacillus* spores at pasteurization temperatures, and stated that more than 99% of spores could be activated at pasteurization temperatures (Phillips and Griffiths, 1933).

Cocci-like bacteria which may form a substantial proportion of the flora of the heat-treated milk grow very slowly at refrigeration temperatures (Rider et al., 1934). *Streptococcus* spp. such as *Streptococcus thermophilus*, *Lactobacillus* *lactis*, and *Streptococcus* *terrestris* which are thermotolerant grow very slowly at refrigeration temperatures. Therefore these bacteria pose no great threat to pasteurized products which

are adequately refrigerated between heat treatment and the ultimate consumer (Johar, 1999).

The quality of pasteurized milk improves with the reduction in levels of post-pasteurization contamination. The majority of the psychrotrophic bacteria are destroyed by pasteurization, and only thermophilic flora, and mesophilic enzymes from psychrotrophic bacteria are left to cause spoilage. The thermostability of proteases, lipases, and phospholipase C of bacterial origin in HTST pasteurization is shown in Table 3-1.

Table 3-1 Bacterial enzyme activity from psychrotrophic organisms in HTST pasteurized milk.

Type of enzyme	Activity after HTST pasteurization (%)
Protease	60
Lipase	70
Phospholipase C	70

Source: Mahi (1990).

About 60% of the protease and lipase activity of the psychrotrophic bacteria remains after HTST treatment, and a lower, but significant, proportion of the phospholipase C activity remains. This thermostability pattern is observed for the proteases of most *Bacillus* and non-*Bacillus* *Pseudomonas* spp. and for enzymes from many species of *Moraxella*, *Acinetobacter*, *Achromobacter*, *Enterobacteriaceae*, and *Morganella*.

Many strains of *Bacillus* spp. are heat resistant, but not their enzymes. Even though growth temperature has a significant effect on the rate of bacterial growth, and on the synthesis of extracellular enzymes, when they are secreted, they exhibit similar degree of thermostability. Psychrotrophic bacteria produce thermostable enzymes almost regardless of species, strain, and temperature of growth. The thermostability of the extracellular enzymes of the psychrotrophic bacteria in milk has a minor importance for short shelf-life products such as pasteurized milk because the shelf life of pasteurized milk is usually about 14 to 30 days, and during this period the products are refrigerated. The total bacterial counts in raw milk for heat treatment is well below the threshold of 10^7 cfu/ml where significant amounts of extracellular degradative enzymes are synthesized (Frank, 1999; Shan, 1999).

UHT MILK

In production UHT milk, the important point is to destroy bacteria while limiting the chemically induced changes such as browning, cooked, and caramelized flavors. UHT milk is either heated in a closed container to yield sterilized milk, or it is heat-treated in a continuous flow, and packed aseptically. The UHT milk is heated at 134-140°C for 2 to 5 sec. The test for sterilized or UHT milk is that samples incubated at 10°C for 15 days will have a plate count of not more than 100 cfu/ml (Brennery and McCance, 1990).

The spore-forming bacteria are the organisms relevant to spoilage in sterilized products, but many species are destroyed by the heat treatment applied to sterilized or UHT milk. Spores of *Bacillus stearothermophilus* constitute the greatest hazard to

spoilage of processed dairy products since they are remarkably heat-resistant, and require extended heat treatments to ensure a reasonable shelf (Mish, 1990).

Residual activity of the extracellular enzymes from psychrotrophic bacteria after UHT sterilisation may be as high as 80% of that in the raw milk. The residual activity of proteases, lipase and phospholipase C are given in Table 3-2. Mish (1990) measured the residual proteases and lipase activity found after heating milk free supernatants at 140°C for 5 sec, and found that *Acetivibrio*, *Stenomonas* and *Shewanella* spp. had residual activities below 10%, and *Flavobacterium pseudomaculatum* had residual activity ranging between 14 and 51%. There could be degradation of milk proteins and fat in the case of a product with a shelf life typically of six months at room temperature (Mish, 1990).

Table 3-2 Residual activity of extracellular enzymes of psychrotrophic organisms after UHT sterilisation

Enzyme type	Residual activity after heat treatment (%)	
	140°C for 5 sec	140°C for 5 sec + 10°C for 1 hr
Protease	20	13
Lipase	40	7
Phospholipase C	9 - 50	n.d.

n.d. = not determined

Source: Mish (1990)

Milk containing different concentrations of a psychrotrophic, proteolytic

Pseudomonas spp. was sterilised, and even though these UHT products were sterile, proteolysis and gelation of the milk occurred. The initial psychrotrophic count affected the shelf life before the onset of gelation. When the microbial count was less than 5×10^2

showed, there was little evidence of an effect on the product quality. However, when the threshold was reached, the decline in shelf life progressed rapidly. There were similar findings when skim-milk with constant increases of 1ml/l of skatol was UHT processed (147°C/2 sec). Premature gelation and bitterness occurred within three months of storage at room temperature. The evidence of smaller defects-capable seen in commercial products from time to time (Blair, 1993). Similar results were observed in the samples from dairy plants and farms, and 70 to 90% of the samples exhibited heat-resistant proteases. After heat treatment at 145°C for 14 sec, these enzymes were still active. It was found that for UHT-sterilized milk to have a shelf life of 1 year, the raw milk must contain less than 0.1 unit of protease/l, and this amount can be easily quantitated within a day for some high-protease-producing bacteria. If acids and whey proteins have been damaged by enzymes of Gram-negative psychrotrophs, they have a predisposition to denaturation and precipitation by UHT treatment. Gelation of UHT milk can occur as a result of the proteolytic activity of these enzymes (Adams et al., 1987).

Lipases can cause fat degradation in UHT products. Acetone affects lipase production by *Pseudomonas* spp. For *P. jugosa*, acetone reduced lipase production, however, a high acetone was required for high lipase activity of *P. aeruginosa* var. *lipolytica* (Champagne et al., 1994).

Milk Volatiles and Off-Flavors

During microbial spoilage, bacteria produce metabolites which cause off-odors. Each type of bacteria has a 'signature' of volatile products that form a unique odor. These

olives can be profiled and/or quantified by sensory and instrumental methods such as gas chromatography.

The possibility of characterizing some bacteria by gas chromatographic analysis of headspace vapors from milk cultures was investigated. Benoit et al. (1987) reported the characteristic patterns and rates of development of acetaldehyde, ethanol, dimethyl sulphide, and dimethyl in cultures of *Lactobacter* spp., *Enterobacter* spp., *Streptococcus flexuatus* var. *spencerensis*, *Selenomonas ruminantium*, *Streptococcus thermophilus*, *S. flexuatus*, *Streptococcus lactis*, *Lactobacillus acidophilus*, *Lactobacillus roreri*, and *Pseudomonas* spp. Dimethyl sulphide, acetaldehyde, 3-methylpentanol, acetone, ethanol, 3-methylbutanol, butanol, 3-methylpentanol-1-ol, and 3-methylpentanol-1-ol in milk cultures of *Streptococcus lactis* var. *spencerensis* were reported (Jelenc et al., 1984). Volatile compounds such as acetone, ethanol, propen-2-ol are produced in refrigerated milk by *Pseudomonas*, and increases in ethanol could be detected when the level of microflora in pasteurized milk stored at 4-10°C reached 10^7 cfu/ml (Kilbuck and Lohse, 1987).

The number of psychrotrophs required to produce off-flavors varies between species, and is determined by the length of the lag period and growth rate at the storage temperature, and by the proteolytic activity and heat resistance of the enzymes. In the case of *Pseudomonas* spp. 1.7×10^7 to 4.3×10^7 cfu/ml and *Alcaligenes* spp. 1.2×10^6 to 3.6×10^6 cfu/ml were required to produce off-flavors. In general, microbial counts at the onset of 1×10^7 cfu/ml are required to produce defects in product quality (Cooms, 1981).

Straw-off-flavors such as bitter, putrid, soapy, rancid, cheesy, yeasty, and sour have been frequently associated with the presence of thermotolerant psychrotrophs in milk. Isolated *Bacillus* spp. were associated only plain milk at a level of 0.2% and incubated at 7.2°C. Their flavor associated defects are shown in Table 3-3.

Table 3-3: Flavor defects associated with psychrotrophic *Bacillus* spp. grown in milk at 7.2°C.

Species	Days to appearance of defect	Flavor defect
<i>B. pasteurii</i>	4-10	Fruity, sour
<i>B. polymyxa</i>	4-8	Sour, yeasty, soapy
<i>B. cereus</i>	4-11	Sweet curdling, soapy, bitter
<i>B. subtilis</i>	2-4	Sweet curdling, bitter
<i>B. lichen</i>	4	Sour
<i>B. coagulans</i>	6	Sweet curdling, bitter
<i>B. globuliformis</i>	11	Soapy, sour

Source: Whittam et al. (1977)

The occurrence of a bitter flavor in milk is related to the presence of heat stable proteins. Hydrolysis of lactalbumin and casein yields bitter peptides. When 500 mg of heat stable proteins from *P. fluorescens* was mixed with pasteurized milk, having low bacterial count, bitterness developed after storage of 7 days at 7°C (Baker, 1963).

Lipases produced by thermotolerant psychrotrophs produce off-flavors in milk. These lipase tends give rise to off-flavors such as rancid, butyric, bitter, soapy, and soapy-sour (Champagne et al.: 1984). The fatty flavor arises from free fatty acid emulsification. Some thermotolerant psychrotrophs produce phospholipases, particularly phospholipase C which is responsible for a specific degradative action on the fat globule

metabolic. The increase in susceptibility of exposed milk to the action of lipase (McLellan, 1949).

Thermolabile psychrotrophs were associated (200 to 100 cfu/ml) into milk whole and skim milk. Sensory defects were observed in the population of 1 to 4×10^7 cfu/ml. This cell population was reached within 6 days at 7°C . Obvious physical and flavor defects were observed when populations of 1 to 20×10^7 cfu/ml in milk were held at 4 to 20°C (Phillips and Goffalo, 1959). The optimum temperature for the production of degradative enzymes by microorganisms is usually lower than the optimum temperature needed for cell division. Even though the microbial population might remain below that normally associated with formation of microbial defects, microbially produced enzymes develop off-flavors when milk is held at refrigeration temperatures for extended periods.

Factors Affecting Shelf Life of Milk

The "shelf life" or "marketable life" is defined as the period between processing/packaging, and when milk becomes unsuitable to consumers (Gillies and White, 1955). The product needs to remain acceptable beyond the last date of sale.

The shelf life of milk is influenced by the quality of raw milk, milk handling, milk processing, and storage temperatures, product processing procedures, and post-pasteurization contamination. Post-pasteurization contamination is most detrimental for keeping quality of pasteurized milk (Singaratz, 1954).

Bacterial growth is responsible for the spoilage of milk, and a level of $\log 7.5$ cfu/ml represents the end of shelf life (Greville et al., 1984). Spoilage represents a case

biochemical changes in the substrate. The amount of substrate utilized, and product formed is proportional to the number of cells present. The substrate is stated as organotrophically spoiled.

When raw milk has a high population of psychrotrophic bacteria ($>10^7$ cells/l), the UHT products obtained from it usually have a reduced shelf life. Collins *et al.* (1982) investigated the influence of the growth of psychrotrophic bacteria in raw milk on the acceptability and residual shelf life of ultra-high temperature (UHT) processed skim milk. The psychrotrophic counts in the raw milk were highly correlated with the extent of proteolysis ($r = 0.97$), however, not with the extent of lipolysis ($r = 0.14$) or the stored UHT milk. It was also found that storage time, and storage temperature had a greater influence on the sensory acceptance and residual shelf life of the UHT skim milk than the psychrotrophic count of the raw milk. Some correlations between the extent of proteolysis and bitterness scores in the stored UHT milk were high at 10°C ($r = 0.70$) and at 40°C ($r = 0.80$), but not at 20°C ($r = -0.13$).

Shelf life prediction could be made by performing microbial enumeration methods, based on proliferation at $15\text{--}20^{\circ}\text{C}$ with or without selective inhibitors, and the results could be obtained after 2-3 days. On the other hand, sensory keeping quality test (7-day score) can be performed, but the result can be obtained after 7-8 days. Due to these time constraints, there is a need for a shelf life test which could provide results in a short period of time, be accurate, simple, and economical to perform (Bishop and White, 1994).

Rapid Methods for the Detection of Microorganisms

The dairy industry experiences increased demands in manufacturing and distribution efficiencies. Food safety and quality management are critical in production and distribution. In order to avoid the sale of contaminated products, expensive investments are held at the production site while samples are tested for microbial contamination, which often takes more than 3 days. Since the products have a short shelf life, they are released before microbial results are available. Rapid detection of pathogens, spoilage microorganisms, and other microbial contaminants in dairy products is important to ensure the safety of consumers and quality of foods.

Recent developments make microbial detection and identification faster, more convenient, more sensitive, and more specific than conventional assays. The most frequently used rapid methods in industry are immunomagnetic separation (IMS), enzyme-linked immunosorbent assays (ELISA) (enzyme for confirmation), bioluminescence, multichannel methods, and other biochemical methods (FDA, Bacteriological Analytical Manual, 1995). These methods may be used on their own or in combination.

Rapid methods have been developed either to replace the enrichment step which requires a prolonged growth period with a concentration step (such as an immunomagnetic separation) or to replace the anti-detection method which is culture dependent that requires a prolonged incubation period (such as an impedance microbiology and bioluminescence).

Immunomagnetic Separation (IMS)

A separation step is normally required in order to discriminate the target organisms from other cells. Superparamagnetic particles are used in immunomagnetic separation. They exhibit magnetic properties in the presence of an external magnetic field. These are coated with antibodies against the target organisms to isolate the organisms selectively.

Food poisoning bacteria can be magnetically separated from foods depending on availability and specificity of appropriate antibodies, but labeled magnetic particles are not available commercially. *E. faecium* and *E. faecalis* from dried and from a poultry (Tancigian, 1992) and *Escherichia coli* serotype from spiked food and water samples (Kaper and Voth, 1993) were isolated using IMS. Encapsulated *S. aureus* can also be isolated from milk using IMS (Johm et al., 1993).

Impedance Microbiology

Impedance microbiology detects microorganisms either directly due to the production of ions from metabolites and products or indirectly from carbon dioxide production. The food market monitor changes in impedance of the growth medium. Microorganisms produce acids and products such as organic acids and ammonia ions from the growth medium, and increase the conductivity of the medium (Sillap and Sivapala, 1996).

In the indirect method, a potassium hydroxide bridge (solidified in agar) is formed across the electrodes. The sample is separated from the potassium hydroxide bridge by a

Respirometry Carbon dioxide accumulation in the headspace during microbial growth. The decrease in the potassium hydroxide. Decrease in conductance occurs once the residual potassium carbonate is less conductive. This method is applicable to a range of microorganisms including *E. aerogenes*, *E. monocytogenes*, *E. faecalis*, *B. subtilis*, *E. coli* & *P. aeruginosa*. *A. hydrophila* and *Salmonella enterica* (Bolton, 1992). This indirect technique is also more appropriate to detect *C. parvulorum* which is a spoilage organism of high pH-values due to its transformation of acid to-carbon dioxide, hydrogen, and isopropanol (Duggan et al., 1983). Impedance microbiology has also been used to monitor the stability of food and biomass starter cultures in the dairy industry (Lazarova et al., 1993).

Enzyme Immunoassays and Ligand Application Tests

The enzyme immunoassay (EIA) or enzyme linked immunosorbent assays (ELISA) are other techniques used in food microbiology. ELISA is performed using microtiter well-bottom coated microtitre strips to capture the target antigen. The captured antigen is detected using antibody antibody which may be conjugated to an enzyme. The presence of the target antigen is revealed by the addition of a substrate (Parryle and Hayes, 1995).

These methods have been developed for the detection of specific pathogens, toxins, and antimicrobials, antibiotics, drugs, and pesticide residues. Some are designed to detect specific organisms such as *Salmonella enteritidis* or *Listeria monocytogenes* from food or environmental samples (Kawachi, 1997). The technique generally requires the

target organism to be more than 10^4 fold). The conventional pre-enrichment and most selective enrichment might be needed prior to testing.

Fluorescent phase latex agglutination (FPLA) can be used to detect *Escherichia coli* enterotoxins. Latex particles coated with specific antisera to the enterotoxins, each on separate particles, are used in the Quick FPLA kit (Dexter Laboratories Ltd.). The sensitivity limit is about 0.5 ng, unconjugated, toxin (Parry and Hayes, 1988).

A number of enzyme immunoassays such as Truen ELISA (Truen Diagnostics) and Vitabid ELISA (bioMérieux) are available for *Escherichia coli* enterotoxin detection. The detection limit for Truen ELISA is less than 0.5 µg (total 100 g food), and requires 7 hrs to obtain the results. Even though the latex agglutination test RPLA has a similar detection limit, it requires 21 hrs to obtain the results (Parry and Hayes, 1988).

The Vitabid (bioMérieux) system contains prepackaged disposable reagent strips. The target organism is captured on a solid phase, receptors coated with primary antibodies, and transferred to the appropriate reagents automatically. This system can be used to detect most major food poisoning organisms (Parry and Hayes, 1988).

Bioluminescent Systems

Bioluminescent systems require the presence of adenosine triphosphate (ATP) in a sample using an enzyme system: luciferase-luciferin, from fireflies. The amount of light generated by this enzymatic reaction can be measured as a variable luminometer, and is directly related to the ATP extracted, and then to the number of microbial cells from which it came (Stanley et al., 1983).

The ATP-bioluminescence method is used to determine milk quality (Van Coillie et al., 1989). The detection level is approximately 1×10^6 cfu/ml of raw milk. Predicting the shelf life of pasteurized milk can also be achieved using ATP-bioluminescence after preincubating the milk at 15°C for 25 hrs or at 21°C for 25 hrs in the presence of crystal violet, penicillin, and streptomycin to inhibit the growth of those positive organisms (Bacteria et al., 1991).

Bioluminescence assays often take just a few minutes to accomplish, but the disadvantages of this system are that the method measures all the bacteria present in the sample, it is not presently possible to selectively measure ATP from one microbial species in the presence of many species (Sawley et al., 1989).

Microarray Methods

Conventional methods for isolation and characterization of microorganisms, especially pathogens, entail the use of special enrichment and cultivation, selective and differential media, and a wide range of biochemical tests. Recently, miniaturized methods, diagnostic kits, and sophisticated instruments have been developed that allow the rapid identification of foodborne pathogens. These methods show an improvement over a conventional test as well as savings in time (Adams and Hope, 1988). The API test (bioMérieux-Vitek), Enterotube (Boehr Diagnostics), Micro-ID system (Organon-Teknica), and Minitek, and Crystal System (BBL Microbiology Systems) are examples of miniaturized kits currently available for use in the food industry. These systems are

convenient, efficient, economical, and easy to use. They are also 95-99% accurate when compared to conventional methods. (Venezuela, 1997)

Other Biomedical Methods

Other biomedical methods include those based on the presence of the lipopolysaccharide of a Gram-negative bacteria (LAM, or *leishman amastigocyte lysate*) and specific enzymes, such as catalase. The LAL test is a simple, rapid, and sensitive method with applications for rapid screening of Gram-negative spoilage bacteria in milk, meats, fish, turkeys, and food ingredients (Venezuela, 1997). This test does not measure the Gram-positive bacteria, so some techniques or devices are needed to relate the LAL-determined Gram-negative bacteria to "total" bacterial number that may be determined by the ratio that exists between Gram-negative and Gram-positive bacteria (Polansky and Rops, 1997). This will increase the cost of the experiment, takes time, and relies on prediction.

In spite of all of these methods, new rapid detection techniques which provide reliable and accurate results in a short period of time that will allow for the performance of effective, corrective measures are needed. In addition to these, a test should be simple and fairly economical. Use of electronic scales may possibly accomplish this. Some results will be given in less than 10-min right after the measurement completed for a qualified user, there is no need for a qualified person to run the experiment, the rest of the experiment will be minimized and small sample size, which is the most important part for some of the food samples, will be enough to perform the experiment.

Electronic Nose

In the food industry, flavors are important from the raw ingredients to the final product. There are two components of flavor perception: taste and aroma. Taste comes from the presence of nonvolatile compounds that interact with sensors in the mouth and on the tongue, and appears as the basic tastes of sweet, sour, salty, and bitter. Although taste is important, the flavor of a food cannot be defined by taste alone.

Many volatile compounds that are responsible for the aroma of a food play an important role in flavor. These volatile compounds contribute to the nature of a food, its product, identity, and to consumer preferences between brands. They are also responsible for the occurrence of off-flavors and taints, which arise because of biochemical or chemical changes, microbial growth or contamination (Hodgson, 1993).

There are three sensory systems in humans that are responsible for the sensation of flavor. These are gustation (sense of taste), olfaction (sense of smell), and the trigeminal sense (responsive to irritant chemical species) (Carlson and Bartlett, 1966).

The sensation of smell depends on the interaction of odor molecules with a group of specialized nerve cells. The odor molecules go into the nasal cavity and across the olfactory mucosa epithelium. These molecules dissolve in an aqueous mucous layer covering the olfactory receptor cells. The olfactory receptor cells located at surface of the olfactory hairs or cilia have receptor binding proteins that bind with the odorant compounds (Jain, 1994; Clayton, 1976; Fennel, 1997). The number of olfactory

receptor cells is large (about 100 million). However, the number of distinct types of binding proteins is small (about 1,000). The same protein has to be found in many different olfactory cells (Barfield et al., 1987). The receptor cells amplify the signal, and transmit it to the brain by means of the axons. The brain compares it with previous knowledge, and tries to define the odor.

Humans can detect a maximum of 10,000 odors, but the number of identifiable odors is approximately 30 (Barfield et al., 1987). The specific combination of complex mixtures of many odorant molecules having different concentrations culminates in the recognizable flavor or odor. Odor molecules are generally small (molecular weight 20-300 Daltons) and polar. They can be detected by humans below 1 ppb. Gas chromatography linked with mass spectrometry (GC/MS) is used to detect complex odors at low levels, but the sample must be separated into its individual components to identify each odor. GC/MS is expensive, and requires a technician for operation and interpretation of the results. Due to these constraints, sensory analysis has been used for a long time for odor evaluation. Sensory analysis has restrictions also: panelists may not be sensitive to some flavors, some raw materials may be difficult to assess using panelists, training needs to be performed before the analysis, sensory analysis can be expensive, and the panelists are subject to fatigue (Barfield et al., 1989; Holgado and Zamora, 1993).

A single molecule can have a distinct odor. However, most natural smells or flavors are a complex mixture of chemical species, and contain hundreds of constituents (Dodd et al., 1993). This aroma is related to the complex interaction of all volatile

compounds within itself. For example, using a gas chromatograph with a sniffing port, some of the individual volatile compounds present in-room smell like roses to humans, however, when all of these compounds combine, the overall aroma is that of roses (Rodgers and Diamond, 1983; Rodgers, 1987).

Due to the limitations of GC/MS and sensory analyses, there has been a need to develop an instrument that can mimic the human sense of smell, and provide rapid sensory information at low cost.

Electronic Nose Technology

In 1983, Moserhoff started to develop an instrument to detect odors. In 1985, several researchers published studies of the relation between the response of elements in an electrode resistance of electrical conductivity and contact potential by elements. The concept of an electronic nose as a chemical array sensor system for odor classification was generated for the first time by Percec and Bond in 1982. Today, the electronic nose has various synonyms such as artificial nose, mechanical nose, mini-robotic system, and sensor array system.

Cooper and Bartlett (1994) defined an electronic nose as an instrument comprised of an array of electronic chemical sensors with partial specificity, and an appropriate pattern recognition system capable of recognizing single or complex odors. The main components of an electronic nose are sample handling mechanism, an array of chemical sensors, signal processing and conditioning, and pattern recognition techniques.

The electronic nose technology simulates the human olfactory process with three sensors, and a suitable software designed to analyse the responses from the sensors. Each sensor represents a group of olfactory receptors, and produces electrical signals in response to an odour, and the electrical signal is time-dependent. Although the specificity of each sensor may be low, the combination of several specificity classes results in a very wide range of information. Any noise or sensor drift may be reduced using signal preprocessing techniques. Finally, the use of pattern recognition in the electronic nose is equivalent to the classification and memorisation of odours in the brain (Gardner and Bartlett, 1999).

The first step in the sample handling is to obtain the vapour above a sample, and to transport it to the sensor array. Currently there are two methods, static and dynamic sampling. In static sampling the headspace above a liquid or solid is measured. The system consists of a sample vessel and a sensor head (compartment with the sensor array). The sensor array and vapour of the sample remain in separate sealed compartments. The sample headspace and sensor head are purged to eliminate any storage before using compressed air or any other inert gas for a certain period of time before the analysis. Once the sample has reached equilibrium, the door between the two compartments is opened and the analysis starts. When conducting polymer sensors are used, an external DC power supply maintains a constant current through the sensors. The sensor resistance changes when the sensors get in contact with the headspace of the sample. The corresponding voltage change across the sensor is measured. The resulting sensing signal is digitised and sent to the computer. The change in conductivity of the sensors is

required for a given period of time. Once the analysis is completed, the measuring loop is closed, and the sensor head and sample vials are purged to get ready for the next analysis.

The electronic nose works similar to a GC in the dynamic sampling procedure. A sample is placed in a closed container. Once headspace equilibration is reached, a sample of the headspace is closed, and injected into the sampling part of the electronic nose. The sample is carried by an inert gas to the sensor array. A sensor changes its electrical properties, and sends a signal to the computer. The test is similar to the static system.

The information provided by the sensor signal is maximized by the signal preprocessing and conditioning of the analog response of the sensor. This is done by using signal conditioning circuits, potential dividers, constant voltage sources, and an analog-to-digital converter (Carmean, 1993). Change in current or voltage is optimized for system sensitivity. Noise is reduced by modulating the sensor signal which is amplified to a suitable level. System noise is affected by variations in the electronic circuitry as well as connections between the sensors and the circuit (Judges, 1995). The signal conditioning improves the response of the sensors, and generates an output that is then analyzed with pattern recognition techniques to define the sample odors.

Sensor Technology

Different types of materials such as conducting polymers, metal oxides, lipid layers, phthalocyanines, and piezoelectric technologies are being used to manufacture sensors that are useful for odor detection. The types of sensors that are being used

commercially or laboratory sensors are semiconductor metal oxides, conducting polymers, quartz resonant sensors, and surface acoustic wave sensors (Barlett et al., 1997, Hodges, 1997). Other types of sensors that have potential or have been used are biosensors, enzyme sensors, electrolyte sensors, plasmas jet wire detectors, and fiber-optic gas sensors (Klauer, 1990). The selectivity and the sensitivity of the sensor are determined by choice of the catalytic surface (Klauer and Barlett, 1988). Sensor technology is changing very rapidly and more sensitive, stable, and fast response sensors are being developed.

Conducting polymers

Conducting polymers used as sensor materials in electronic noses have unique electrical properties that make them suitable for gas detection. A wide range of materials can be synthesized. They respond to a broad range of organic vapors, and they operate at room temperature. The main types are poly-pyrrole and poly-aniline. The volatile compounds change the electrical conductivity of the polymer. This change occurs rapidly and reversibly at room temperature (Klauer and Barlett, 1990). The adsorbed odor molecules are believed to cause a swelling of the polymers and to interfere with charge transfer within the polymer (Klauer, 1993). Conducting polymer sensors are nonspecific. Different compounds will interact with the polymer material. These sensors are small, and have low power consumption since they operate at room temperature. They have quite a good sensitivity, typically between 1 l and 100 ppm (Barlett et al., 1997). The sensor responses are site-specific with rapid recovery of the baseline when the

volatile compound is removed. Conducting polymers are sensitive to humidity, therefore, caution should be taken when analysing samples with different water activities.

Semiconductor metal oxide chemoresistive sensors

These types of sensors are developed from chemoresistive arrays of inorganic semiconducting materials such as oxides, and analytic metals. Two main types have been developed: thick film metal oxides, known as Tapesol sensors, and thin films, which are commonly used in commercial electronic noses. These sensors comprise a substrate support tube containing a platinum heater coil. The substrate is coated on the outside of the sensor support tube along with the analytic metal oxides such as palladium or platinum. An current passes through the coil, the metal oxide heats up. The reaction between the vapor and the metal oxide causes a change in electrical resistance at a fixed temperature. This resistance change is to be measured, and related to a substance being monitored. In metal oxides, chemisorbed oxygen (O^*) reacts with the polymer (R) reversibly to produce oxidized materials (RO_2) and liberated conducting electrons (e^-). Electron mobility increases, and electrical conductivity of the material changes (Tao et al., 1995). These sensors operate between 200–300°C to avoid interference from water, and to aid rapid response and recovery times (Karlsson and Karlsson, 1995). They are sensitive to combustible materials, such as alcohols, but are less sensitive at detecting sulfur- or nitrogen-based odors.

Surface acoustic wave devices

Surface acoustic wave sensors have been at research and development for 5 to 10 years (Hodges, 1997). The principle of operation is that a surface wave is generated as a

material that directs the compounds of interest. The surface wave is normally generated using a quartz resonator, and the frequency of operation is usually between 100 MHz and 1 GHz. The frequency of operation depends on the sensitivity required by the system. When the sensor is not exposed to a vapor, it will have a certain resonant frequency. When the sensor is in contact with the volatile, there will be a change of mass in the sensor material, and therefore, a change in the resonant frequency (Bakker and Wubbles, 1989; Hodgins, 1997). This frequency change is the response or output from the sensor to the volatile present in the sample analyzed. These sensors have higher sensitivity than conducting polymers (Hodgins, 1997). However, they are more selective, and a larger number of these sensors are needed to cover all vapors that are likely to occur in food products.

Fiber-optic gas sensors

These sensors rely on the light guiding properties of the optical fiber to carry the light from the light source to the chemically sensitive layer, and then to return the light to the sensor. The optical properties measured include the optical path length, birefringence, absorption, fluorescence, and reflectance.

These types of sensors have potential advantages: the individual fibers can be as small as 1 μ m in diameter, and large bundles of fibers are available which permit an attractive approach to the fabrication of massive sensor arrays; video technology can be used to measure the response from an array; the measurements can be made remotely because the fiber allows transmission of light over long distances, and the devices are not subject to electrical interference (Gardner and Bartlett, 1999).

In recent studies, some researchers have shown that fiber-optic gas sensor arrays can be used to detect a range of organic vapors (Dickinson et al., 1996; White et al., 1996). A fluorescent dye, Nile Red, was used in these studies, since the fluorescence spectrum, and intensity of Nile Red is strongly dependent on the local solution environment of the dye molecule. The fluorescence intensity from the dye changes in the presence of organic vapors that sorb into the polymer film. This change can be measured. At present, the sensitivity of these sensors is not high, and there is not enough information about the lifetime, reproducibility, or stability of fiber-optic sensors.

Applications of Electronic Nose

The electronic nose is used for monitoring and control of industrial processes, diagnosis in the medical field, environmental control, and for control of food quality. It is possible to classify various liquors, perfumes, tobacco brands, beers, and many more with this device.

In the environmental applications, the electronic nose was used in monitoring sewage related odors. Canonical correlation was used to compare the multivariate data generated by the electronic nose (Miniature Olfactory Sensing Equipment) with sensory panel analysis. A linear relationship can be obtained between the electronic nose data and the corresponding threshold odor numbers within the similar groups of data from the experiment (Jinuta et al., 1991). In another application, the odors from pig and chicken slurry were evaluated by using a photoacoustic detector and electronic nose based on polypyrrole sensors, and it was concluded that electronic nose was better at discriminating

between different odors through the pattern of sensor responses (Hobbs et al., 1993). In this case rapid and portable devices for odor measurement may be useful since some of the major odor compounds were chemically unstable.

In the paper industry, an electronic nose containing four Tagoide gas sensors, and one sulfured-CDs, sensor was used to measure the odors from five cardboards papers from commercial manufacturers. It was shown that the olfactory quality of cardboard papers could be measured using an electronic nose (Kobayashi et al., 1999).

Two different electronic noses (Noseworks-e-Nose 4000SM equipped with conducting polymer sensors and Alpha M O S Pro 3000SM equipped with metal oxide sensors) were used to distinguish between different concentrations of two food flavor mixtures, ten different methanol samples, and nine tobacco samples. Both instruments distinguished between different concentrations of the same flavor for two different flavors for a given day. Ten methanol samples were distinguished from each other for a given day based on sensory analysis. Nine tobacco sample results showed discrimination between each other that were classified as good, borderline, or bad using both instruments (Hobbs, 1993).

Applications in Microbial Detection

Given the sensitivity of the electronic nose, it has great potential in microbiological analysis. Gordon and Cervera (1994) reported on the use of a 4-element metal oxide system to discriminate between 4 types of bacteria (*Clostridium perfringens*, *Proteus*, *Enterococcus faecium*, *Starcococcus faecalis*, *Cytophaga*, *Pseudomonas*

arrangement). An array of 4 Taguchi series-4 gas sensors was used to sample the headspace of the 4 different bacterial samples and a control effluent agar. The data was used to train a neural network by the backpropagation method using over 1,000 iterations with a set of 42 odor vectors. The results were reasonably encouraging with 64.3% of test vectors being correctly classified.

Two bacteria types, *Enterobacter aerobius* and *Sophophomonas aeruginosa*, were also examined. A backpropagation network was trained on the 90 odor vectors and as 87% of the test vectors the age of the bacteria were correctly classified. Another backpropagation network was trained on 140 vectors to predict the bacteria type, and as 81% of the 160 test vectors the type of bacteria of any age was correctly classified (Gardner and Cronin, 1994). Electronic nose was successfully used to separate *Pseudomonas* species which produce different volatile metabolites (Olsson et al., 1995).

The electronic nose and neural network classifier were also used to detect and simultaneously identify pure plate cultures of a range of microorganisms. The overall classification rate for 12 different bacteria (*E. Coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Bacillus cereus*, *Edwardsiella aerogenes*, *Sophophomonas aeruginosa*, *Sophophomonas sporobius*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella paratyphi*, *Bacillus subtilis*), and one pathogenic yeast (*Candida albicans*) was 83-89%. Three similar pure cultures were also compared, and the correct classification rate was 94.3%. Principal component analysis gave good discrimination between water vapor and the test organisms (Olsson et al., 1997).

In medical applications the electronic nose is used to scan wounds to diagnose infection and monitor the healing process. A hospital in Manchester, England has shown that the nose can detect early signs of wound infection, and can even distinguish between different infecting organisms. Electronic nose is also used in the analysis of breath of subjects. Researchers at the University of Pennsylvania detected pneumonia in hospitalized patients by collecting breath samples, and analyzing by electronic nose (Hudson, 1997).

Applications in Food Products

The applications of an electronic nose in the food industry are virtually unlimited. The instrument is currently used in the identification and classification of different food products based on their color.

In most food companies raw materials are not checked frequently for aroma before processing. Final product is checked, and the batch is rejected if a taste or off-color is present. This may cause difficulty in determining which supplier delivered the faulty material, and the faulty material may have cost a great deal to the manufacturer (Hodgson, 1997). However, if the company uses the electronic nose as a quality tool to check raw materials, the problem can be eliminated.

Electronic nose can also be used to monitor food colors during critical stages of production to ensure that optimum processing conditions are being maintained, to monitor product deterioration during shelf life studies and during transport to retailers. It is concluded that the system is ideal for quality (CIRQA) checking (Hodgson, 1997).

In the dairy area, sensor arrays have been used to discriminate the role of fatty acids in the aroma profile of Swiss cheese (Hager et al., 1996), and to differentiate organoleptically modified cheese varieties (Jin and Harper, 1998). Karel et al. (1999) used the electronic nose to detect the odor differences in milk due to microbial load, storage time, and sensory panel perception. Shreeveghen et al. (1998) also obtained the selective discrimination of different heat treated commercially available milks (pasteurized, UHT, and sterilized) with an array of four semiconductor sensors. The sensors were formed by sputtered semiconductor thin film materials. The volatiles from the milk samples were collected by a dynamic headspace and directly directed into the test chamber where the sensor array was placed. The data was processed with principal component analysis. They stated that the results were promising for the industrial development of an electronic nose for the monitoring of the milk quality.

In the meat area, electronic nose was used for separation of ground meat and cooked samples of pork-beef mixtures according to composition and freshness (Turhan et al., 1998), and used for estimation of quality of ground meat, stored under a polyethylene sheet, and gave a good possibility of predicting storage time (Wolpin et al., 1993). Electronic nose was also used to monitor sausage fermentation following the changes in volatiles during the fermentation process, and to compare the electronic nose results with sensory panel results. From the sensor readings the fermentation time could be predicted, and sensory panel results were compared with the electronic nose sensor readings at the early stage of the process and at the final samples (Hilde et al., 1998).

In the earlier years, applications of the electronic nose have been done in differentiation of odors in dairy-based on its (Baldwin and Luning, 1994), storage of rice (Nirwan, 1994), and wheat (Luning and Baldwin, 1995a) at different temperatures using conducting polymer sensors. Electronic nose can also used to monitor humidity and soil moisture (Dahsen et al., 1992), and to determine fish storage time (de Nante et al., 1990). The odor of decomposition in raw and cooked shrimp was evaluated based on electronic nose readings, sensory evaluation, and ammonia levels (Luning and Baldwin, 1995b).

The fruits and vegetables area has also benefited from the electronic nose. The electronic nose has been successfully employed for determination of harvest ripeness in watermelons (Denny et al., 1994), and quality assessment of peeled mushrooms (Nason et al., 1995). Volatile of citrus juice (Hedgren, 1993; Hedgren and Sauerbault, 1994), and fresh-squeezed orange juice aroma volatiles (Bazzucchi et al., 1996) have been studied. Bazzucchi et al. (1997) reported that grapefruit juices of different cultivars were discriminated using metal oxide sensors. Masi et al. (1997) assessed the ability of an electronic nose to nondestructively identify and classify tomato fruit exposed to different harvesting and postharvest handling treatments. Werling and Wickstrom (1997) compared the sensory quality of conventionally processed carrots, green beans, and potatoes using metal oxide sensors and sensory panels.

In the case of grains and beans, Ekstrom et al. (1996) used an electronic nose to classify grains, and therefore reduce the inspection exposure to grains that can be contaminated with aflatoxins. Jonsson et al. (1997) analyzed samples of rice, rye, and

barley with different colors and wheat with different levels of ergosterol, fungal, and bacterial loads. The color of classes of good, moldy, weakly, and strongly moldy oats was predicted with a degree of accuracy using artificial neural networks. The percentage of moldy barley or rye grains in the mixture of fresh grains was also indicated. It was also reported that there was a high degree of correlation between artificial neural network predictions and measured ergosterol, fungal, and bacterial loads. Hoffmann et al. (1997) used an electronic nose equipped with 4 metal oxide semiconducting sensors to follow the flavor generation during roasting of wheat bread, and to follow the roasting degree of toasted wheat bread slices. Some other work has been also done to discriminate among coffee cultivars, coffee from different origins, and coffee species (Andriani, 1991; Tan et al., 1993; Delmon et al., 1994).

In non-alkoholic and alcoholic drinks area there is a significant potential for the use of electronic noses. The flavor and aroma of beer and its raw materials were monitored using electronic nose technology (Peters et al., 1995; Tominson, 1995; Tominson et al., 1995; Ziemann and Loderer, 1995). The aroma of pure hops and blends used in beer making were studied by Lucas and Caron (1991) and Weber and Pabst (1994). Viana and Bartolotti (1994) also used an electronic nose to help in the determination of the technical specifications of some additives and technological aids used in the sparkling wine process.

Since the electronic nose is rapid and objective in quantifying odors, there is a great potential in quality-control applications in various worldwide

Current Shortcomings of the Electronic Nose

In electronic nose technology, the individual sensors need to be reproducible in their response to a given odor or chemical. It is important that a given sensor is reproducible throughout the lifetime of that sensor. However, this is no simple problem for electronic nose applications due to the difficulties of multivariate calibration, and the complex data sets that are required to train the pattern recognition software. The problem with the reproducibility of response over time arises from drift in the sensitivity of the sensors and the poisoning effects. Drift is a slow change in sensitivity, occurs with time, and can also be due to the effects of aging, slow morphological changes in the sensor material and some other long-term effects. Sensor poisoning occurs when a sensor is exposed continuously to a material which irreversibly binds to, or otherwise with, the sensing material leading to a reduction or even total loss of sensitivity. It is possible to avoid the poisoning problem by carefully selecting the right type of sensor for the particular application and by avoiding poisons. Unfortunately, there is no chemical sensor which cannot be poisoned.

Another aspect of reproducibility is the response reproducibility between sensors of nominally the same type. This is important because if a sensor is poisoned then it can be replaced without recalibration and retraining the system. If the sensors are sufficiently reproducible in their response, then it becomes possible to train one sensor array, and to use the same training set for any nominally identical array in other instruments at different locations. If the irreproducibility of data from one electronic nose to another (given

manufacture) can be obtained, the use of electronic nose in quality control applications can spread to various worldwide.

Changes in temperature, humidity and flow rate also play an important role in sensitivity. These effects can be minimized by careful system design and sample handling, but this makes the electronic nose more expensive and complex (Kilian et al., 1998). However, it is necessary to overcome the effects of changes in temperature and humidity on the sensor's baseline and on the magnitude of their responses before portable instruments become available.

Objectives

The overall objectives of this study were to determine the ability of an electronic nose and sensory panels to detect the presence of pure cultures of *P. fluorescens* or *B. cereus* in sterilized whole, reduced-fat, and fat-free milk samples stored at 1 °C, 7.2°, and 12 °C, and, to determine the ability of an electronic nose to predict the shelf life of milk. The specific objectives were:

1. To monitor whole, reduced-fat, and fat-free milk samples with known microbial loads of *P. fluorescens* or *B. cereus* stored the samples at 1 °C, 7.2°, and 12 °C, and measure the electronic nose sensor response at 0, 3, 7, 1, and 35 days in storage,
2. To conduct an odor sensory panel to determine whether the panels can detect the difference between a reference sample, and inoculated stored samples.

- 3 To determine and statistically correlate the relationship between electronic nose readings and the microbial numbers for *P. fluorescens* or *B. coagulans* inoculated into whole, reduced-fat, and fat-free milk, stored at 1 °C, 7 °C, and 12 °C,
- 4 To determine and statistically correlate the relationship between electronic nose readings and the sensory evaluations for whole, reduced-fat, and fat-free milk inoculated with *P. fluorescens* or *B. coagulans* stored at 1 °C, 7 °C, and 12 °C,
- 5 To attempt to predict shelf-life (based on microbial numbers) using electronic nose readings from accelerated studies,
- 6 To inoculate whole milk samples with known microbial loads of *P. fluorescens* and *B. coagulans*, store the samples at 1 °C, 7 °C, and 12 °C, and measure the electronic nose sensor responses at 0, 3, 5, 8, and 10 days in storage,
- 7 To conduct an odor sensory panel to determine whether the panelists can detect the difference between a reference sample and stored samples inoculated with both microorganisms,
- 8 To determine and statistically correlate the relationship between electronic nose readings and the sensory evaluations for whole milk inoculated with both microorganisms, stored at 1 °C, 7 °C, and 12 °C.

CHAPTER 1 MATERIALS AND METHODS

Milk Sampling, Incubation, and Analysis

Milk Samples

Whole, reduced-fat (2% milkfat) and fat-free milk (Formula[®], Danone, NJ) was purchased from a local supermarket in Gainesville, FL. The samples were aseptically packaged in 146 ml packages and the sell-by-date and the lot numbers for each set of samples were the same. The packages were kept at 4 °C until the experiments.

The sampling procedures were performed under a sterile laminar flow hood (Nasco Biological Safety Cabinets, Plymouth, MN). The front surface of the milk package was modified thoroughly with 70% alcohol. A sterile, single use FreononGlate[®] needle (B-D[®] 18 G T5 Barium Dickinson and Company, Co. No. 307158, Franklin Lakes, NJ) w/ a sterile, single use 60 ml syringe (B-D[®] Boston Dickinson and Company Co. No. 3094581, Franklin Lakes, NJ) was inserted through the previously modified package wall. A 50-ml milk sample was taken from the package, placed into a 100-ml sterile jar and the lid was closed.

Activation of Microorganisms

The milk samples were inoculated with *Pseudomonas fluorescens* (ATCC 944) (American Type Culture Collection, Manassas, VA) and/or *Acetivibrio conjugatus* (ATCC 7953). *P. fluorescens* represents the Gram-negative spoilage organism in milk and *A. conjugatus* for Gram-positive microflora. The freeze-dried culture of *P. fluorescens* was activated by adding some of the culture into 10 ml nutrient broth (Difco-Laboratories, Cat. No. 0000-01-4, Detroit, MI) tube and incubated at 21°C for 48 hrs. The nutrient broth with the activated organism was transferred to 150 ml nutrient broth flask and incubated at 21°C for 48 hrs. The freeze-dried culture of *A. conjugatus* was activated by adding some of the culture into 10 ml tryptic soy broth (Difco Ltd., Cat. No. C01 016, Hampshire, England) and incubated at 21°C for 48 hrs. The tryptic soy broth with the activated organism was transferred to 150 ml tryptic soy broth flask and incubated at 21°C for 48 hrs.

The activated organisms were centrifuged by using a Sorvall® RC-3B refrigerated superspeed-centrifuge (Du Pont Corp., Duluth, GA) at 4040 x g for 10 min for *P. fluorescens* and at 1000 x g for 10 min for *A. conjugatus*. The supernatant was poured off and the cultures were resuspended with filter sterilized phosphate buffer saline (Harrison, 1946). They were centrifuged at 4040 x g and 1200 x g for 10 min for *P. fluorescens* and *A. conjugatus*, respectively. The procedure was repeated twice. Twenty ml of Bacterfield's buffer solution (International BioProducts Inc., Richmond, VA) was added to each centrifuge tubes. Eleven ml of the slusp in from each centrifuge were

transferred to 99 ml Butterfield's buffer dilution bottle. This dilution was used for all the inoculations of *P. ovipalens*. One more dilution was performed for *P. fluorescens*, 1 ml from the previous dilution bottle was transferred to 99 ml Butterfield's buffer dilution bottle, and this dilution was used for all the inoculations.

Inoculation of *Cholera parvum* and *Salmonella typhimurium*

The jars filled with 50 ml of milk were inoculated with 1 ml of the specific dilution for each microorganism and the control samples were inoculated with 1 ml of Butterfield's buffer solution. The samples were stored at 1 °C, 1 °C, and 12.8 °C for up to 10 days. The samples were evaluated at days 0, 1, 3, 5, 7, and 10.

The samples for the accelerated study were prepared similarly, but the samples were stored at 1 °C for up to 4 days. At day 0, five jars for each treatment were inoculated at 12 °C for 24 hrs and these samples were analysed at day 1. At days 3, 5, and 7, five jars for each treatment were taken out from the 1 °C refrigerator and incubated at 12 °C for 24 h and they were analysed at days 4, 6, and 8. This study was repeated twice for each type of milk using milk from the same lot.

Whole milk (*primula*®, Denmark, NE) was used for the confirmation study. In this study, the milk samples were inoculated with 1 ml of each *P. fluorescens* and *P. ovipalens* dilutions. The control samples were inoculated with 1 ml of Butterfield's buffer solution. The storage conditions and the analyses were the same as in the other studies.

Electronic Nose Measurements

An electronic nose (e-Nose) 4000 model, EFT Inc., Amford, NJ, equipped with twelve conducting polymer sensors (sensor types: 411, 478, 484, 491, 492, 493, 495, 496, 497, 498, 499, 500) was used to quantify the sensor responses to differences in odor of milk samples inoculated with different microorganisms. The electronic nose measurements were done immediately (day 0), and at days 1, 3, 4, 5, 6, 7, 8, and 10. Five replicates were analyzed by the electronic nose for each treatment. Each replicate was kept at room temperature for 30 min prior to analysis in order to let the milk temperature equilibrate to room temperature (22.5° to 23.5°C). Five replicates were analyzed on each day for each storage temperature. The replicates were flushed with compressed air for about 15 sec prior to the electronic nose analysis. The milk jar was placed in the glass sampling vessel of the electronic nose. One day before the experiment started, the electronic nose was calibrated with 75% w/v propylene glycol solution (100% solution from Fisher Scientific, Inc. P-115-10, Fair Lawn, NJ), following the manufacturer's recommendations. Every day before the experiments the electronic nose was turned on and compressed air (CO2A Grade D, Brite Writing Supply Inc., Jacksonville, FL) was passed through the sensors for at least 30 min. The vessel was purged with compressed air for 2 min to eliminate any foreign odor present in the vessel from the environment for each replicate, and then the sensor head was purged for 4 min with compressed air. During these 4 min, the sample volatiles were equilibrating in the headspace of the vessel. Sensor response data was acquired for 4 min. Total analysis time for each milk sample took 10

not. Readings at 4 sec exposure of the sensors to the milk samples were used for data analysis. At the end of the day, electronic test sensors were cleaned with compressed air for at least 30 min. Electronic test raw-data can be obtained from Dr. Mingsi Shieh, Food Science and Human Nutrition Department at the University of Florida (E-mail: "Mshieh@ufl.edu").

Microbial Analysis

Microbial counts were performed for each container at each sampling day. Dilutions were made using pre-filled sterile disposable Bacteroid's buffer dilution bottles. Total aerobic count method using Petrifilm technique was used to enumerate *part.F*. *Stenotrophomonas* count was the only microflora present. Insulated aerobic plate count Petrifilm (3M Company, St. Paul, MN) were incubated at 30°C for 48 hrs.

Barbey and Juntz (1988) used agar pour plates and Petrifilm dry medium culture plates to enumerate bacteria after preliminary incubation of milk samples at 11°C or 21°C for 18 hrs. Results showed that the Petrifilm technique was not significantly different from agar pour plate methods. Gossel et al. (1994) reported that total aerobic bacteria data comparisons obtained by the Standard Plate Count and Petrifilm method produced a correlation of 0.95 and 0.94, respectively. Since the results of the Petrifilm technique and agar pour plate methods are similar, the Petrifilm technique may be preferred because they require no preparation, and are ready to use.

Part.E. *campylobacter* cultures were enumerated using modified plate count agar (APIC) (Difco Ltd., Cat. No. CM93, Hampshire, England) by spread plate method

They were incubated at 32°C for 48 hrs. Colonies were counted and reported as \log_{10} cfu/ml.

Microbial counts for incubated samples of milk was performed as follows: 4 ml of milk from one milk jar and 1 ml milk from another jar of the same treatment were taken and transferred to the same dilution bottle to ensure homogeneity. From pre-determined dilutions, 1 ml was taken and plated in the Petri film for *P. fluorescens*. They were incubated at 32°C for 48 hrs. For *S. conjugans* 0.1 ml from pre-determined dilutions was spread plated onto standard plate count agar and incubated at 32°C for 48 hrs. A 1 ml sample was taken from control samples, placed in Petri film and incubated at 32°C for 48 hrs. Microbial counts were performed on the control samples to see whether there was any contamination occurred during filling the jars, and any contaminated sets were discarded. All sets used for data analysis were free of contamination. Colonies were counted and reported as \log_{10} cfu/ml of milk.

For the confirmation study, 4 ml and 1 ml milk samples from two replicates were transferred to the dilution bottles. Serial dilutions were performed and 0.1 ml was spread plated onto crystal violet tetrasolium agar (Marshall, 1994) and thermomaculorum agar (Difco Laboratories, Cat. No. 6450-17-1, Detroit, MI) and incubated at 32°C and 37°C for 48 hrs, respectively. Crystal violet tetrasolium agar was used to enumerate *P. fluorescens*, and thermomaculorum agar was used to enumerate *S. conjugans*. For control samples, 1 ml milk sample was plated in Petri film and incubated at 32°C for 48 hrs. Colonies were counted and reported as \log_{10} cfu/ml of milk.

Moisture Content Measurements

Moisture content was measured in triplicate for each type of milk and reported twice using the oven method (Bradley et al., 1982). This analysis was performed to have information on the composition of the milk in order to better describe the sample. A sample of approximately 3 g was placed in an aluminum weighing dish (50 mm diameter, Co. No. 08-710, Fisher Scientific, Fair Lawn, NJ). The sample was placed in an oven at 100°C for 24 hrs. Moisture content was reported as percent wet basis.

Fat Content Measurements

Fat content was measured according to Chaffard et al. (1991). This analysis was done to confirm that each type of milk had the same fat percentages as it was stated on the labels. Two ml of ethanol, 0.3 ml of 12.1 N HCl, and 25 ml of hexane were added to 5 ml of milk. The mixture was shaken and centrifuged at 500 x g for 3 min. The top organic layer was transferred to a clean tube, and the aqueous phase was reextracted with 25 ml of hexane. Digests tubes were placed in the same tube and water was removed with sodium sulfate, and the solvent evaporated under a stream of nitrogen. The total fat percentage was determined gravimetrically.

pH Measurements

A 10 ml sample of milk in the jar was placed in a clean plate. The pH electrode (ORION pH electrode, Model 11-41, Orion Research Inc., Beverly, MA) was connected to an Expanded Ion Analyzer, and was calibrated every day with pH 4.00 and 7.00 standards.

(buffer solution pH 4.00, SR 01-500 and pH 7.00, SR 07-500, Fisher Scientific, Fair Lawn, NJ). Measurements were performed for each treatment at every time interval and in duplicate.

Sensory Evaluation

The odor of milk samples was evaluated by a 12-member trained sensory panel consisting of students, 21-32 years of age, from the Food Science and Human Nutrition Department at the University of Florida. A difference-threshold test was performed at days 0, 1, 3, 7, and 10. Panelists were asked to smell milk samples and denote if there was any difference in odor among the treated samples and the reference sample. The reference sample was fresh *Panadol*[®] milk from the same lot used for the experiments. Panelists rated the difference as 0 to 10 scale (0 = no difference and 10 = very different). Samples were randomized and a hidden control was included in the test. The replicate number 1 was always taken out of the refrigerator 30-min before the sensory analysis at each day. All panelists smelled the same samples. Sensory tests were carried out in both experiments and in the combination study.

Data Analysis

Electronic nose sensor readings were analyzed in Statistica for Windows (16 editions, Statsoft Inc., Tulsa, OK) using discriminant function analysis (DFA) as reported by other researchers (Cousens, 1993; Gardner and Hogg, 1993; Gardner and Barber, 1995). Multicollinearity and sensory data were used as grouping variables and 12 electronic nose sensor outputs were used as independent variables. DFA was used to

develop predictive models for classification of samples based on grouping variables. The 13 sensor outputs were reduced to 2 discriminant functions. These functions were used to map the data in two dimensional plots and observe separation between groups. Correct classification rates and the coefficients for each function were calculated using Statistics.

Data of the compositional analysis, microbiological analysis, pH measurements and sensory scores for each treatment were subjected to analysis of variance with the general linear model (GLM, IBM). Least square means were obtained and reported using the least significant difference test procedure when significant ($p < 0.05$) if values were obtained.

CHAPTER 4 RESULTS AND DISCUSSION

Milk Sampling, Incubation, and Analysis

Molasses and Fat Content Measurements

The molasses contents of whole, reduced-fat (2% milkfat), and fat-free milk samples were $39.33\% \pm 0.11$, $90.18\% \pm 0.26$, and $93.26\% \pm 0.22$, respectively. The fat contents of whole, reduced-fat, and fat-free milk samples were $3.23\% \pm 0.01$, $1.93\% \pm 0.01$, and $0.11\% \pm 0.01$, respectively. As the molasses content increased, the fat content decreased.

Microbial Analysis

Microbial counts for *E. faecium* and *S. aureus* during 10 days of storage for each type and two experiments of milk samples are given in Tables 4.1 and Appendixes A for whole, reduced-fat and fat-free milk. Analysis of variance with the general linear model procedure was performed for each incubated microorganism and for each experiment of each type of milk to see if there was any significant difference due to the storage temperature and time. It was expected that the storage temperature and time would have an effect on the microbial load. It was found that the storage temperature,

Table 4.1 Microbial load of all types of milk samples inoculated with *Pseudomonas fluorescens* or *Bacillus coagulans*.

Storage Time (Days)			Microbial Load (log ₁₀ cfu/ml of milk) ^a					
			Experiment 1			Experiment 2		
			17°C	5.2°C	12.4°C	17°C	5.2°C	12.4°C
Whole Milk	Inoculated with <i>Pseudomonas fluorescens</i>	0	4.52 ^a	4.28 ^a	4.28 ^a	3.90 ^a	3.60 ^a	3.90 ^a
		1	4.12 ^a	4.50 ^a	4.20 ^a	5.20 ^a	5.00 ^a	3.70 ^a
		2	3.43 ^a	7.20 ^a	3.80 ^a	6.03 ^a	4.10 ^a	6.00 ^a
		3	4.40 ^a	7.20 ^a	5.20 ^a	4.00 ^a	5.00 ^a	5.20 ^a
		4	4.30 ^a	5.00 ^a	5.20 ^a	4.70 ^a	4.43 ^a	5.20 ^a
		10	4.30 ^a	5.00 ^a	5.20 ^a	4.70 ^a	4.43 ^a	5.20 ^a
	Inoculated with <i>Bacillus coagulans</i>	0	4.50 ^a	4.50 ^a	4.50 ^a	3.90 ^a	3.60 ^a	3.90 ^a
		1	2.50 ^{abc}	1.40 ^b	1.20 ^{abc}	4.60 ^{abc}	4.40 ^{abc}	4.10 ^{abc}
		2	2.40 ^{abc}	1.30 ^b	2.10 ^{abc}	4.20 ^a	3.00 ^{abc}	3.20 ^a
		3	1.80 ^b	2.60 ^{abc}	1.10 ^b	3.20 ^{abc}	4.70 ^{abc}	3.70 ^a
		4	2.60 ^a	2.60 ^a	2.60 ^a	3.40 ^a	4.40 ^{abc}	4.10 ^{abc}
		10	2.60 ^a	2.60 ^a	2.60 ^a	3.40 ^a	4.40 ^{abc}	4.10 ^{abc}
Reduced-fat Milk	Inoculated with <i>Pseudomonas fluorescens</i>	0	3.12 ^a	3.20 ^a	3.20 ^a	3.40 ^a	3.40 ^a	3.40 ^a
		1	3.00 ^a	3.20 ^a	4.40 ^a	3.60 ^a	3.60 ^a	3.60 ^a
		2	3.20 ^a	3.20 ^a	5.20 ^a	3.80 ^a	3.80 ^a	5.10 ^a
		3	4.20 ^a	4.20 ^a	5.20 ^a	4.10 ^a	4.40 ^a	5.20 ^a
		4	4.20 ^a	4.20 ^a	5.20 ^a	4.10 ^a	4.40 ^a	5.20 ^a
		10	3.20 ^a	3.20 ^a	5.20 ^a	4.40 ^a	3.60 ^a	5.20 ^a
	Inoculated with <i>Bacillus coagulans</i>	0	3.12 ^a	3.20 ^a	3.20 ^a	3.10 ^a	3.10 ^a	3.10 ^a
		1	3.20 ^{abc}	4.00 ^a	3.40 ^a	3.00 ^a	3.40 ^a	3.00 ^a
		2	4.40 ^a	4.40 ^a	4.10 ^a	3.80 ^a	3.80 ^a	3.80 ^a
		3	3.80 ^a	3.80 ^a	4.80 ^a	4.20 ^a	3.20 ^a	3.20 ^a
		4	3.20 ^a	3.20 ^a	4.20 ^a	4.20 ^a	3.20 ^a	4.20 ^a
		10	3.20 ^a	3.20 ^a	4.20 ^a	4.20 ^a	3.20 ^a	4.20 ^a
Fat-free Milk	Inoculated with <i>Pseudomonas fluorescens</i>	0	3.00 ^a	3.00 ^a	3.00 ^a	4.10 ^a	4.20 ^a	4.10 ^a
		1	3.12 ^a	4.10 ^a	3.00 ^a	4.00 ^a	3.00 ^a	3.70 ^a
		2	4.20 ^a	4.40 ^a	4.00 ^{abc}	4.20 ^a	4.20 ^a	5.10 ^a
		3	4.20 ^a	4.20 ^a	5.10 ^a	4.20 ^a	4.20 ^a	5.10 ^a
		4	4.20 ^a	4.20 ^a	5.10 ^a	4.20 ^a	4.20 ^a	5.10 ^a
		10	3.12 ^a	4.00 ^a	4.10 ^a	3.70 ^a	3.70 ^a	4.60 ^a
	Inoculated with <i>Bacillus coagulans</i>	0	3.40 ^a	3.40 ^a	3.40 ^a	3.80 ^a	3.80 ^a	3.70 ^a
		1	3.40 ^a	2.80 ^{abc}	3.70 ^a	3.80 ^a	4.20 ^a	4.20 ^a
		2	3.40 ^a	3.60 ^a	3.60 ^a	3.40 ^a	3.80 ^a	4.60 ^a
		3	3.20 ^{abc}	3.20 ^a	2.20 ^{abc}	3.00 ^a	3.40 ^a	3.00 ^a
		4	3.20 ^a	3.20 ^{abc}	2.50 ^{abc}	3.00 ^a	3.40 ^a	3.00 ^a
		10	3.20 ^a	3.20 ^{abc}	2.50 ^{abc}	3.00 ^a	3.40 ^a	3.00 ^a

^a Averaging 1 of two readings

^{abc} Superscripts indicate no significant for each microorganism (three significant difference at the p<0.05). Means were separated using LSD

time and the interactions were significant ($P<0.05$) for each microorganism and for each experiment of each type of milk.

The microbial loads for *P. fluorescens* were plotted versus storage time and are shown in Figures 4.1-4.3 for each type of milk and for each experiment. In general *P. fluorescens* counts increased gradually during storage at 1 °C, but they increased rapidly at 12.8°C. The growth of *P. fluorescens* at 7.2°C showed a similar trend with the ones at 12.8°C except for whole milk experiment 1 and reduced-fat milk experiment 2. In whole milk experiment 1 and reduced-fat milk experiment 2, there were lag phases until day 3 and then exponential growth occurred. This could be due to the differences of inoculation loads. Regardless of the initial inoculation levels or the storage temperature, *P. fluorescens* counts exceeded 10^7 cfu/g at day 10 for all types of milk. Since microbial counts in the range of 1×10^6 cfu/g are required to produce obvious defects in product quality (Cousa, 1982), formation of off-odors was expected during storage and these were detected by electronic nose.

The microbial loads for *B. cereus* were plotted versus storage time and are shown in Figures 4.4-4.6 for each type of milk and for each experiment. The initial inoculation levels were between 10^2 and 10^3 cfu/g. However, this microorganism was unable to grow at 1 °C, 7.2°C and 12.8°C. The microbial counts were generally lower for the milk samples stored at 1 °C than the samples stored at 7.2°C and 12.8°C except for reduced-fat milk experiment 1. The micro-organisms isolated as thermotolerant psychrotrophs in the literature and these may be variants of mesophilic organisms that have adapted to grow at lower temperatures (Gruzekopf and Harper, 1974). *B. cereus* used in this study

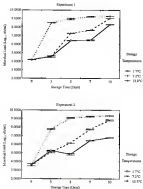


Figure 4-1 Average microbial loads of *F. fluorescens* for whole milk, both experiments stored at different temperatures over time. Error bars signify ± 1 standard deviation.

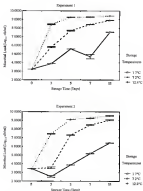
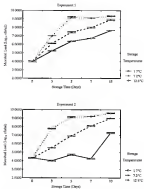


Figure 4-1 Average microbial load of *P. fluorescens* for reduced-fat milk, both experiments stored at different temperatures over time. Error bars signify ± 1 standard deviation.



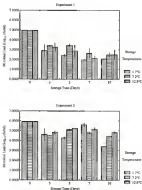


Figure 4-4 Average recorded load for *A. coarctatus* for whole cells, both experiments stored at different temperatures over time. Error bars signify ± 1 standard deviation.

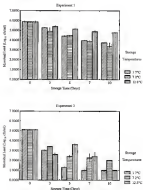


Figure 4-5 Average normalized load for *A. complanatus* for reduced fat cells, both experiments stored at different temperatures over time. Error bars signify ± 1 standard deviation.

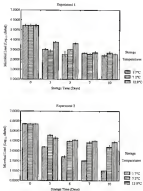


Figure 4-4 Average microbial load for *B. cereus* for ice-free milk, both experiments stored at different temperatures over time. Error bars signify ± 1 standard deviation.

(ATCC 2890) which was isolated from an exposed milk soon probably lost its ability to grow at refrigeration temperatures. Attempts were made to adapt the microorganism to grow at low temperatures. Initially, it was subcultured in tryptic soy broth at 31°C and was transferred to a new sterile tryptic soy broth and incubated at 21°C. Milk samples were inoculated with this and the organism was expected to grow at lower temperatures, but it did not proliferate at refrigeration temperatures.

Accelerated Study

The microbial loads for all milk types, both experiments and two microorganisms for the accelerated study are given in Table 4.2. The microbial counts for *L. florissantis* increased approximately to 10^7 cfu/ml for all milk types after 24 hrs of incubation at 28°C. The initial inoculation level had an effect on the microbial growth. Whole milk experiment 1 was inoculated with the microbial load of $4.20 \log_{10}$ cfu/ml and the microbial load increased to $8.32 \log_{10}$ cfu/ml after 24 hrs at 28°C. On the other hand, whole milk experiment 2 was inoculated with $3.60 \log_{10}$ cfu/ml and the microbial growth reached to $7.94 \log_{10}$ cfu/ml. This was observed in all types and experiments of milk.

The *L. florissantis* counts increased to 10^7 , 10^8 cfu/ml for day 4, 5, and 6. Due to the possibility of insufficient substrate, the microbial loads did not increase more than 10^8 cfu/ml throughout the accelerated study regardless of the microbial load of the milk sample that was inoculated at each time period. The type of milk did not have a significant effect on the microbial loads for this study.

The microbial counts for *B. cereus* increased for all milk types after each 24 hrs of incubation at 28°C. At day 6, the initial microbial load was $4.20 \log_{10}$ cfu/ml for

Table 4-2 Microbial load of all types of milk inoculated with *Pseudomonas fluorescens* or *Bacillus coagulans* in condensed milk^a

Milk Type	Analysis Time (Days)	Microbial Load (\log_{10} cfu/ml of milk) ^a			
		Experiment 1 (Inoculated <i>Micrococcaceae</i>)		Experiment 2 (Inoculated <i>Micrococcaceae</i>)	
		<i>P. fluorescens</i>	<i>B. coagulans</i>	<i>P. fluorescens</i>	<i>B. coagulans</i>
Whole Milk	0	8.02	4.58	7.64	4.58
	4	8.74	3.48	8.13	3.73
	8	8.79	3.48	8.70	3.48
	8	9.29	3.78	8.43	4.48
Reduced-Fat Milk	0	7.88	7.04	7.64	7.47
	4	8.77	8.07	8.77	8.73
	8	8.88	7.85	8.81	8.78
	8	8.75	7.09	8.80	7.40
Fat-free Milk	0	8.42	7.79	8.23	8.08
	4	8.58	7.38	8.46	7.38
	8	9.08	7.43	8.43	7.00
	8	8.02	6.43	8.23	7.08

^a Average of two readings

whole milk experiment 1 and it increased to 4.38 log₁₀ cfu/ml after incubation. Even though only a slight increase in microbial number was observed in this case, in other cases, higher increases in the microbial loads were observed. For example at day 7 the microbial load for the first milk experiment 2 increased 1 log cycle after the incubation at 20°C. This also proved that the organism has not been adapted to grow at low temperatures.

On the other hand, it was observed that *B. coagulans* counts for all types and experiments of milk samples were decreased over time. The milk samples were kept at 1 °C before the accelerated study analysis, and the microorganisms could not survive at low refrigeration temperatures as mentioned before. The types of milk had a significant effect in this study, but this could be observed because of not having the same initial inoculation levels at day 0 for each type and experiment of milk samples.

Combination Study

In the combination study, *P. fluorescens* and *B. coagulans* counts are given in Table 4-1. The storage time and temperature had significant effects on the microbial loads for both microorganisms. *P. fluorescens* counts increased rapidly for the samples stored at 12.8°C (Figure 4-7) and they increased gradually for the samples stored at 7.2°C until day 7, and had a rapid increase from day 7 to day 10. The microbial loads for *P. fluorescens* increased to 10⁷ cfu/ml for the samples stored at 7.2° and 12.8°C at day 10. On the other hand, *P. fluorescens* in the whole milk samples stored at 1 °C multiplied, but did not show any increase in counts compared to the samples stored at the higher temperatures. This could occur due to the presence of *B. coagulans* together with *P. fluorescens* in the samples.

Table 4-3 Microbial load of whole milk samples inoculated with *Paratuberculosis* *fluorescens* and *Bacillus coagulans* at combination study

Storage Time (Days)	Microbial Load (log ₁₀ cfu/ml of milk) ^a					
	<i>Paratuberculosis fluorescens</i> Storage Temperature			<i>Bacillus coagulans</i> Storage Temperature		
	1 °C	7 °C	12 °C	1 °C	7 °C	12 °C
0	3.80 ^a	3.80 ^a	3.80 ^a	3.80 ^a	3.80 ^a	3.80 ^a
1	3.08 ^a	3.40 ^a	7.49 ^b	4.40 ^a	5.15 ^b	5.37 ^b
3	3.40 ^a	4.15 ^a	6.77 ^b	4.54 ^a	5.29 ^b	6.30 ^b
7	3.80 ^a	4.55 ^a	7.08 ^b	4.64 ^a	4.84 ^b	6.94 ^b
10	3.70 ^a	4.80 ^a	9.20 ^b	7.40 ^b	4.40 ^b	4.90 ^b

^a Average of two readings

^b Superscript within each row-signifies denote significant difference at the p=0.05. Means were separated using LSD.

The microbial loads for *B. coagulans* for the samples stored at each storage temperature decreased during the 10-day storage. However, for the samples stored at 7 °C and 12 °C, the decrease in *B. coagulans* counts was less compared to the decrease in the counts for the samples stored at 1 °C (Table 4-3 and Figure 4-5). This was due to the lack of adaptation of *B. coagulans* at low refrigeration temperatures.

Application Study of the Combination Study

The microbial loads for *P. fluorescens* and *B. coagulans* for the accelerated study of the whole milk samples in the combination study are given in Table 4-4. After each incubation at 28 °C, *P. fluorescens* counts were increased to 10⁷ cfu/ml. *B. coagulans* counts increased during incubation compared to the microbial load of the sample before it was incubated. However, *B. coagulans* counts decreased over time.

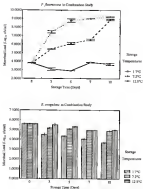


Figure 4-7 Average microbial load for *P. fluorescens* and *B. megaterium* for combination study of whole milk, stored at different temperatures over time. Error bars signify ± 1 standard deviation.

Table 4-4 Microbial load of whole milk samples inoculated with *Paratuberculosis* (fluorescent) and *Bacillus cereus* as spoiler in accelerated study

Storage Time (Days)	Microbial Load (log ₁₀ cfu/ml of milk)*	
	<i>Paratuberculosis</i> (fluorescent)	<i>Bacillus cereus</i>
1	8.34	8.69
4	7.83	8.30
6	8.45	8.64
8	8.26	8.83

* Average of two readings

pH Measurements

The pH of all whole, reduced-fat and fat-free milk samples stored at different temperatures during 18 days of storage are given in Tables 4-3, 4-4, and 4-5, respectively (Appendix B). Changes in the pH of whole, reduced-fat and fat-free milk during storage did not follow any specific trend. The pH of each type of milk and the experiments of each milk was significantly different ($p < 0.05$). Therefore data for each milk type and experiments of these were analyzed independently. Overall, pH of the samples inoculated with *P. fluorescens* decreased during storage. This decrease was higher for the samples stored at 12.8°C than for the samples stored at 1.7°C and 7.2°C, except for the fat-free experiment 2 samples stored at 7.2°C and 12.8°C, where the pH increased. However, changes in pH were not as large as microbial loads. In most cases, there were maximum pH changes for control and samples inoculated with *B. cereus*.

Table 4-1 pH of whole milk inoculated with *Pseudomonas fluorescens* or *Acetivibrio* *compositus*

Storage Time (Days)			pH								
			1°C			7°C			12°C		
			C	Pf	Pc	C	Pf	Pc	C	Pf	Pc
Exp. 1	0	Avg. ^a	6.73	6.73	6.73	6.73	6.73	6.73	6.73	6.73	6.73
		St. dev.	0.06	0.04	0.08	0.06	0.01	0.01	0.06	0.03	0.01
	3	Avg.	6.68	6.74	6.64	6.73	6.75	6.66	6.58	6.53	6.67
		St. dev.	0.01	0.13	0.04	0.01	0.04	0.01	0.03	0.02	0.01
	5	Avg.	6.67 ^b	6.80 ^a	6.70 ^b	6.67 ^b	6.77 ^a	6.67 ^b	6.77 ^a	6.67 ^b	6.76 ^b
		St. dev.	0.01	0.04	0.05	0.01	0.01	0.01	0.04	0.02	0.00
	7	Avg.	6.62 ^b	6.77 ^a	6.70 ^b	6.70 ^b	6.70 ^a	6.60 ^b	6.67 ^a	6.57 ^b	6.60 ^b
		St. dev.	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.04	0.01
	10	Avg.	6.70 ^b	6.70 ^b	6.70 ^b	6.70 ^b	6.67 ^a	6.70 ^b	6.77 ^a	6.77 ^a	6.76 ^b
		St. dev.	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.01
	0	Avg.	6.76	6.76	6.76	6.76	6.76	6.76	6.76	6.76	6.76
		St. dev.	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01
	3	Avg.	6.76	6.75	6.76	6.75	6.67	6.73	6.73	6.73	6.74
		St. dev.	0.00	0.01	0.00	0.00	0.11	0.03	0.01	0.00	0.00
Exp. 2	0	Avg.	6.74	6.73	6.73	6.73	6.73	6.76	6.75	6.68	6.67
		St. dev.	0.07	0.08	0.00	0.00	0.00	0.00	0.01	0.01	0.01
	7	Avg.	6.77 ^a	6.70 ^b	6.60 ^b	6.70 ^b	6.70 ^b	6.77 ^a	6.77 ^a	6.60 ^b	6.60 ^b
		St. dev.	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01
	10	Avg.	6.74 ^a	6.70 ^b	6.70 ^b	6.70 ^b	6.70 ^b	6.70 ^b	6.70 ^b	6.60 ^b	6.67 ^b
		St. dev.	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01

Avg.^a Average of two readings

St. dev. Standard deviation of the means

C Control samples

Pf Samples inoculated with *P. fluorescens*

Pc Samples inoculated with *A. compositus*

[a] Superscripts within a row denote significant difference at the p<0.05. Means were separated using LSD

Table 4-1 pH of rehydrated milk inoculated with *Parabacterium flavescens* or *Bacillus coagulans*

Storage Time (Days)		pH									
		1 °C			12 °C			22 °C			
		C	PF	Bc	C	PF	Bc	C	PF	Bc	
Exp. 1	0	Avg. ^a	6.75	6.76	6.75	6.75	6.76	6.75	6.75	6.76	6.75
		St. dev.	0.00	0.02	0.01	0.00	0.11	0.01	0.00	0.11	0.01
	2	Avg.	6.72 ^a	6.70 ^a	6.70 ^a	6.72 ^a	6.70 ^a	6.70 ^a	6.73 ^a	6.69 ^a	6.70 ^a
		St. dev.	0.06	0.00	0.00	0.01	0.10	0.01	0.00	0.00	0.01
	5	Avg.	6.79 ^a	6.76 ^a	6.76 ^a	6.79 ^a	6.76 ^a	6.77 ^a	6.79 ^a	6.77 ^a	6.76 ^a
		St. dev.	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.00
	7	Avg.	6.77 ^a	6.69 ^a	6.77 ^a	6.77 ^a	6.60 ^b	6.77 ^a	6.77 ^a	6.77 ^a	6.77 ^a
		St. dev.	0.01	0.01	0.00	0.01	0.08	0.01	0.00	0.00	0.01
	10	Avg.	6.78 ^a	6.69 ^a	6.70 ^a	6.78 ^a	6.60 ^b	6.70 ^a	6.78 ^a	6.69 ^b	6.70 ^a
		St. dev.	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01
Exp. 2	0	Avg.	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
		St. dev.	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00
	3	Avg.	6.76	6.78	6.78	6.73	6.78	6.78	6.77	6.68	6.68
		St. dev.	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01
	5	Avg.	6.70 ^a	6.77 ^a	6.76 ^a	6.70 ^a	6.77 ^a	6.77 ^a	6.74 ^a	6.69 ^a	6.70 ^a
		St. dev.	0.01	0.00	0.01 ^a	0.01	0.01	0.00	0.00	0.01	0.00
	7	Avg.	6.70 ^a	6.70 ^a	6.75 ^a	6.70 ^a	6.70 ^a	6.70 ^a	6.74 ^a	6.74 ^a	6.70 ^a
		St. dev.	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
	10	Avg.	6.67 ^a	6.67 ^a	6.71 ^a	6.67 ^a	6.77 ^a	6.66 ^a	6.77 ^a	6.66 ^a	6.67 ^a
		St. dev.	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01

Avg.^a Average of two readings.

St. dev. Standard deviation of the means.

C: Control samples

PF: Samples inoculated with *P. flavescens*

Bc: Samples inoculated with *B. coagulans*

^{a,b} Superscripts within a row denote significant differences at the p=0.01. Means were separated using LSD.

Table 4-5 pH of fat-free milk inoculated with *Pseudomonas fluorescens* or *Bacillus subtilis*

Storage Time (Days)		pH								
		1 °C			7 °C			12 °C		
		C	PF	B ₁₂	C	PF	B ₁₂	C	PF	B ₁₂
Exp. 1	0	Aug.	6.71	6.78	6.78	6.77	6.78	6.78	6.75	6.74
		St. dev.	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	3	Aug.	6.73	6.74	6.73	6.73	6.74	6.73	6.76	6.76
		St. dev.	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.03
	8	Aug.	6.72 ^a	6.72 ^a	6.71 ^a	6.70 ^a	6.70 ^a	6.70 ^a	6.72 ^a	6.71 ^a
		St. dev.	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.05
	7	Aug.	6.63	6.73	6.74	6.73	6.76	6.72	6.68	6.71
		St. dev.	0.05	0.03	0.03	0.03	0.03	0.04	0.03	0.03
	10	Aug.	6.74	6.67 ^a	6.74	6.73	6.73	6.74	6.67 ^a	6.72
		St. dev.	0.03	0.11	0.03	0.03	0.03	0.03	0.03	0.03
Exp. 2	0	Aug.	6.67 ^a	6.70 ^a	6.67 ^a	6.67 ^a	6.70 ^a	6.67 ^a	6.70 ^a	6.67 ^a
		St. dev.	0.03	0.04	0.03	0.03	0.03	0.03	0.04	0.03
	3	Aug.	6.72 ^a	6.72 ^a	6.73 ^a	6.74 ^a	6.73 ^a	6.73 ^a	6.73 ^a	6.73 ^a
		St. dev.	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.03
	8	Aug.	6.71	6.71	6.74	6.71	6.70	6.71	6.70	6.70
		St. dev.	0.05	0.04	0.03	0.03	0.03	0.03	0.03	0.03
	7	Aug.	6.67 ^a	6.70 ^a	6.67 ^a	6.70 ^a	6.71 ^a	6.67 ^a	6.71 ^a	6.67 ^a
		St. dev.	0.03	0.04	0.03	0.03	0.03	0.03	0.04	0.03
	10	Aug.	6.70 ^a	6.70 ^a	6.70 ^a	6.70 ^a	6.70 ^a	6.70 ^a	6.70 ^a	6.70 ^a
		St. dev.	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

^aAug. Average of two readings.

St. dev. Standard deviation of the means.

C: Control samples.

PF: Samples inoculated with *P. fluorescens*.

B₁₂: Samples inoculated with *B. subtilis*.

^aSuperscript letters in row denote significant difference at the p<0.05. Means were separated using LSD.

pH Measurements for Accelerated Study

In the accelerated study, the changes in pH for all types of milk during storage did not follow any specific trend (Table 4-8). According to the 10-day storage study, pH for the samples inoculated with *P. fluorescens* and stored at higher temperatures decreased more than for the samples stored at lower temperatures. However, the opposite occurred in the accelerated study. The incubation temperature for the accelerated study was 38°C and it was expected to have higher pH drops, but contrary to this, pH increased for all milk types and experiments except for whole milk experiment 1 samples. The reason behind this was not understood. In most cases, there were slight pH changes for control and samples inoculated with *B. coagulans*. No research has been found in the literature on effects of growth of *P. fluorescens* and *B. coagulans* on pH.

pH Measurements for Combination and Accelerated Studies

In the combination study, pH values for whole milk control and inoculated with *P. fluorescens* and *B. coagulans* samples are given in Table 4-9. The pH values of all control samples at all storage temperatures increased. On the other hand, pH values for samples inoculated with both microorganisms and stored at 1 °C did not change, but pH values for samples stored at 7 °C and 10 °C decreased. The reason for this could not be explained.

The pH values for the whole milk control and inoculated with *P. fluorescens* and *B. coagulans* for the accelerated study are given in Table 4-10. For the control samples, pH slightly dropped, but pH values increased for the other samples.

Table 4-8 pH of all types of milk inoculated with *Parabacterium*/*Neobacterium* or *Exiguobacterium* in accelerated study

Milk Type	Analysis Time (Days)	pH					
		Experiment 1 (Inoculated microorganisms)			Experiment 2 (Inoculated microorganisms)		
		Control	<i>Parabacterium</i> <i>Neobacterium</i>	<i>Exiguobacterium</i>	Control	<i>Parabacterium</i> <i>Neobacterium</i>	<i>Exiguobacterium</i>
Whole Milk	1	Aug.*	6.64	6.61	6.71	6.74	6.71
		St. dev.	0.01	0.01	0.05	0.01	0.01
	4	Aug.*	6.71	6.61	6.66	6.74	6.61
		St. dev.	0.01	0.01	0.04	0.01	0.01
	8	Aug.*	6.86	6.84	6.77	6.78	6.78
		St. dev.	0.01	0.01	0.06	0.01	0.01
	12	Aug.*	6.94	6.84	6.87	6.84	6.71
		St. dev.	0.04	0.01	0.01	0.01	0.01
Reduced Fat Milk	1	Aug.*	6.71	6.74	6.70	6.71	6.70
		St. dev.	0.01	0.01	0.01	0.01	0.01
	4	Aug.*	6.81	6.71	6.69	6.74	6.70
		St. dev.	0.01	0.01	0.01	0.01	0.01
	8	Aug.*	6.90	6.70	6.81	6.84	6.71
		St. dev.	0.01	0.01	0.01	0.01	0.01
	12	Aug.*	6.80	6.71	6.64	6.71	6.74
		St. dev.	0.04	0.04	0.01	0.01	0.01
Fat-free Milk	1	Aug.*	6.66	6.66	6.44	6.55	6.54
		St. dev.	0.04	0.04	0.01	0.01	0.01
	4	Aug.*	6.54	6.54	6.50	6.71	6.55
		St. dev.	0.04	0.01	0.04	0.01	0.01
	8	Aug.*	6.41	6.70	6.69	6.74	6.64
		St. dev.	0.01	0.01	0.01	0.01	0.01
	12	Aug.*	6.71	6.61	6.71	6.74	6.71
		St. dev.	0.01	0.01	0.01	0.01	0.01

Aug.* Average of two readings

St. dev. Standard deviation of two readings

Table 4-9 pH of whole milk control and inoculated with *P. fluorescens* and *B. coagulans* samples

Storage Time (Days)	Sample	pH					
		Control			<i>Pseudomonas fluorescens</i> and <i>Bacillus coagulans</i>		
		Storage Temperature			Storage Temperature		
		12°C	22°C	32°C	12°C	22°C	32°C
0	Avg. ^a	6.63	6.65	6.65	6.50	6.50	6.50
	St. dev.	0.00	0.00	0.00	0.01	0.01	0.00
3	Avg.	6.66	6.67	6.67	6.50	6.50	6.50
	St. dev.	0.00	0.00	0.00	0.00	0.00	0.00
6	Avg.	6.66	6.70	6.71	6.54	6.55	6.57
	St. dev.	0.00	0.00	0.01	0.04	0.00	0.04
9	Avg.	6.70	6.71	6.70	6.53	6.55	6.56
	St. dev.	0.01	0.01	0.00	0.00	0.00	0.00
10	Avg.	6.70	6.70	6.71	6.50	6.54	6.54
	St. dev.	0.00	0.00	0.00	0.00	0.01	0.01

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Table 4-10 pH of whole milk control and inoculated with *P. fluorescens* and *B. coagulans* samples in acidified milk

Analysis Time (Days)	Sample	pH	
		Control	<i>Pseudomonas fluorescens</i> and <i>Bacillus coagulans</i>
1	Avg. ^a	6.70	6.68
	St. dev.	0.00	0.00
4	Avg.	6.68	6.68
	St. dev.	0.00	0.00
6	Avg.	6.71	6.70
	St. dev.	0.00	0.00
9	Avg.	6.70	6.70
	St. dev.	0.01	0.00

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Sensory Evaluation

Sensory data showed that in general panellists detected the odor differences due to the growth of *P. fluorescens* in all types of milk samples, but had difficulties in detecting the odor changes due to *B. coagulans*. This might be because *B. coagulans* could not grow at 1 °C, 7 °C, and 12 °C and did not generate enough volatile metabolites which gave off-odors. Sensory scores given by six panellists for all milk types are given in Appendix C. The average sensory scores for whole, reduced-fat, and fat-free milk control, inoculated with *P. fluorescens* or *B. coagulans* and hidden control samples and their standard deviations are shown in Tables 4-10, 4-11 and 4-12, respectively. The hidden control samples were the same as the reference samples. At day 0, hidden control samples were not presented to the panellists since all the samples were fresh and they all were assumed to have a difference of 0.

The average sensory scores for all milk types and for both experiments were rounded off to the nearest integer to facilitate analysis using DFA. These scores are given in Table 4-14. In most cases, especially toward the end of the storage period and at higher storage temperatures, the sensory scores for control and hidden control samples are significantly different from the sensory scores of samples treated with *P. fluorescens* or *B. coagulans*. The scores for samples treated with *P. fluorescens* were significantly different from the rest of the sensory scores. Overall, the sensory scores for the controls and hidden-control samples were not significantly different from each other. As the temperature and storage time increased, the sensory scores given to the samples

Table 4-11 Summary means for whole milk associated with *P. fluorescens* or *B. cephalus*

Inoculated Microorganisms	Storage Time (Days)	Sensory Scores					
		Fragrance			Taste		
		1 (FC)	2 (FC)	3 (FC)	1 (TC)	2 (TC)	3 (TC)
Control	0	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	1	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	2	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	7	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	10	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	15	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
<i>Pseudomonas fluorescens</i>	0	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	1	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	2	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	7	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	10	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	15	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
<i>Bacillus cephalus</i>	0	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	1	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	2	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	7	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	10	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
	15	Avg. *	0.00	0.00	0.00	0.00	0.00
		St. dev.	0.00	0.00	0.00	0.00	0.00
Whole/Control	0	Avg. *	-	-	-	-	-
		St. dev.	-	-	-	-	-
	1	Avg. *	-	0.00	0.00	-	0.00
		St. dev.	-	0.00	0.00	-	0.00
	2	Avg. *	-	0.00	0.00	0.00	0.00
		St. dev.	-	0.00	0.00	0.00	0.00
	7	Avg. *	-	0.00	0.00	0.00	0.00
		St. dev.	-	0.00	0.00	0.00	0.00
	10	Avg. *	-	0.00	0.00	0.00	0.00
		St. dev.	-	0.00	0.00	0.00	0.00
	15	Avg. *	-	0.00	0.00	0.00	0.00
		St. dev.	-	0.00	0.00	0.00	0.00

The 1 to 3 scale was used (0 = no difference and 3 = very different)

Avg. * Average of ten readings

St. dev. Standard deviation of ten readings

Table 4-12. Sensory scores for reduced-fat milk emulsified with *P. fluorescens* or *B. coagulans*.

Emulsified Microorganisms	Storage Time (Days)	Sensory Scores					
		Appearance			Taste		
		1-3%	1-4%	1-6%	1-3%	1-6%	11-6%
Control	1	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	3	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	5	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	7	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	10	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
<i>Pseudomonas fluorescens</i>	1	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	3	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	5	Aug.	3.50	3.48	3.50	3.47	3.50
		St. dev.	0.00	0.01	0.00	0.01	0.00
	7	Aug.	3.50	3.48	3.50	3.51	3.50
		St. dev.	0.00	0.01	0.00	0.01	0.00
	10	Aug.	3.50	3.48	3.50	3.43	3.50
		St. dev.	0.00	0.01	0.00	0.03	0.00
<i>Bacillus coagulans</i>	1	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	3	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	5	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	7	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
	10	Aug.	3.50	3.50	3.50	3.50	3.50
		St. dev.	0.00	0.00	0.00	0.00	0.00
Hobbs/Control	1	Aug.	-	-	-	-	-
		St. dev.	-	-	-	-	-
	3	Aug.	-	3.50	3.50	3.50	3.50
		St. dev.	-	0.00	0.00	0.00	0.00
	5	Aug.	-	3.50	3.50	3.50	3.50
		St. dev.	-	0.00	0.00	0.00	0.00
	7	Aug.	-	3.50	3.50	3.50	3.50
		St. dev.	-	0.00	0.00	0.00	0.00
	10	Aug.	-	3.50	3.50	3.50	3.50
		St. dev.	-	0.00	0.00	0.00	0.00

The first 10 trials were used for \bar{x} - no difference and \bar{x} - very different.

Aug. = Average of two readings.

St. dev. = Standard deviation of two readings.

Table 4-13 Summary scores for 60-day milk inoculated with *P. fluorescens* or *B. coagulans*

Inoculated Microorganisms	Storage Time (Days)	Sensory Scores					
		Experiment 1			Experiment 2		
		1, 2%	1, 5%	11, 12%	1, 2%	1, 5%	11, 12%
Control	0	Apr. 8.50	8.50	8.50	8.50	8.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	1	Apr. 8.50	8.50	8.50	8.50	1.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	1.00	0.00
	2	Apr. 7.50	7.50	7.50	8.50	1.50	8.50
		St. dev. 1.00	0.00	0.00	0.00	1.00	0.00
	7	Apr. 8.50	7.50	7.50	8.50	1.50	8.50
		St. dev. 0.00	1.00	0.00	0.00	1.00	0.00
	10	Apr. 8.50	7.50	7.50	8.50	0.50	8.50
		St. dev. 0.00	1.00	0.00	0.00	0.00	0.00
	15	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
<i>Pseudomonas fluorescens</i>	0	Apr. 8.50	8.50	8.50	8.50	8.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	1	Apr. 8.50	8.50	8.50	8.50	1.50	8.50
		St. dev. 0.00	1.00	0.00	0.00	1.00	0.00
	2	Apr. 7.50	7.50	7.50	7.50	1.50	7.50
		St. dev. 0.00	0.00	0.00	0.00	1.00	0.00
	7	Apr. 8.50	7.50	7.50	7.50	1.50	7.50
		St. dev. 0.00	1.00	0.00	0.00	1.00	0.00
	10	Apr. 8.50	7.50	7.50	8.50	1.50	8.50
		St. dev. 0.00	1.00	0.00	0.00	1.00	0.00
	15	Apr. 8.50	7.50	7.50	8.50	0.50	8.50
		St. dev. 0.00	1.00	0.00	0.00	0.00	0.00
<i>Bacillus coagulans</i>	0	Apr. 8.50	8.50	8.50	8.50	8.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	1	Apr. 8.50	8.50	8.50	8.50	1.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	1.00	0.00
	2	Apr. 8.50	8.50	8.50	8.50	1.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	1.00	0.00
	7	Apr. 7.50	7.50	7.50	7.50	1.50	7.50
		St. dev. 0.00	0.00	0.00	0.00	1.00	0.00
	10	Apr. 7.50	7.50	7.50	7.50	1.50	7.50
		St. dev. 0.00	0.00	0.00	0.00	1.00	0.00
	15	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
Hidden/Control	0	Apr. -	-	-	-	-	-
		St. dev. -	-	-	-	-	-
	1	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	2	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	7	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	10	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00
	15	Apr. 8.50	8.50	8.50	8.50	0.50	8.50
		St. dev. 0.00	0.00	0.00	0.00	0.00	0.00

The 0-to 10 scale was used (0 = no difference and 10 = very different).

Apr. = Average of two readings

St. dev. = Standard deviation of two readings

Table 4-14 Average sensory scores (standard) of all types of milk inoculated with *P. fluorescens* or *P. casei* at 4°C

Milk Type	Storage Time (Days)	Sensory Scores											
		1°C				4°C				11°C			
		C	PF	Ba	Hc	C	PF	Ba	Hc	C	PF	Ba	Hc
Whole Milk	Rep 1	0 Avg.	8	0	0	0	0	0	0	0	0	0	0
		2 Avg.	1	1	1	1	1	1	0	1	1	1	0
		3 Avg.	2	2	2	1	1	1	1	2	2	2	2
		4 Avg.	2	2	1	1	2	2	2	2	2	2	2
		10 Avg.	1	1	1	1	2	2	2	2	2	2	2
		0 Avg.	0	0	0	0	0	0	0	0	0	0	0
	Rep 2	1 Avg.	1	1	1	1	2	2	2	2	2	2	1
		2 Avg.	2	2	2	1	2	2	1	2	2	2	2
		3 Avg.	1	2	1	1	2	2	1	1	2	1	2
		10 Avg.	1	2	1	1	2	2	1	2	2	2	2
		0 Avg.	0	0	0	0	0	0	0	0	0	0	0
		2 Avg.	0	0	0	0	0	0	0	0	0	0	0
Reduced-fat Milk	Rep 1	0 Avg.	1	2	2	1	2	2	1	2	2	2	2
		2 Avg.	1	2	2	1	2	2	2	2	2	2	2
		3 Avg.	1	2	2	1	2	2	2	2	2	2	2
		4 Avg.	1	2	2	1	2	2	2	2	2	2	2
		10 Avg.	1	2	2	1	2	2	2	2	2	2	2
		0 Avg.	0	0	0	0	0	0	0	0	0	0	0
	Rep 2	1 Avg.	1	1	1	1	2	2	1	2	2	1	2
		2 Avg.	2	2	2	2	2	2	2	2	2	2	2
		3 Avg.	1	1	1	2	2	2	2	2	2	2	2
		4 Avg.	1	1	2	2	2	2	2	2	2	2	2
		10 Avg.	1	1	2	2	2	2	2	2	2	2	2
		0 Avg.	0	0	0	0	0	0	0	0	0	0	0
Part-fat Milk	Rep 1	0 Avg.	0	0	0	0	0	0	0	0	0	0	0
		2 Avg.	0	0	1	0	1	1	1	2	2	2	2
		3 Avg.	2	2	2	2	2	1	2	2	2	2	2
		4 Avg.	2	2	2	2	2	2	2	2	2	2	2
		10 Avg.	2	2	2	2	2	2	2	2	2	2	2
		0 Avg.	0	0	0	0	0	0	0	0	0	0	0
	Rep 2	1 Avg.	1	1	1	0	1	1	2	2	2	2	2
		2 Avg.	2	2	2	2	2	2	2	2	2	2	2
		3 Avg.	2	2	2	2	2	2	2	2	2	2	2
		4 Avg.	2	2	2	2	2	2	2	2	2	2	2
		10 Avg.	2	2	2	2	2	2	2	2	2	2	2
		0 Avg.	0	0	0	0	0	0	0	0	0	0	0

The 0 to 10 scale was used (0 = no diff. and 10 = very different)

Avg. = Average of two readings

C = Control samples

PF = Samples inoculated with *P. fluorescens*

Ba = Samples inoculated with *B. casei*

Hc = Within control samples

^a Superscripts in each row within each storage temperature denote significant difference at the $p \leq 0.1$. Means were separated using LSD.

associated with *P. fluorescens* increased which meant that the colors of those samples were getting very different than the color of the reference sample which was fresh milk. This was as expected since when the microbial counts exceeded 10^7 cfu/ml, the product started to have off-flavors. This caused an adverse influence on product quality.

Sensory data for the whole milk inoculated with both microorganisms in the continuation study also showed that the panelists detected the overall color changes caused by the microorganisms. The sensory scores given by ten panelists are given in Appendix C.

C. The average sensory scores and their standard deviations for the continuation study are shown in Table 4-13. The average sensory data in Table 4-13 was rounded off to the nearest integer to facilitate analysis with DFA as shown in Table 4-14. Panelists rated the control and holder control samples from 0 to 1. There were no significant differences between these two samples according to the panelists except for the samples stored at 7 °F and 13 °F at day 10. They rated the control samples as 1 and holder control samples as 0 (Table 4-14). The mean and difference mean ratings were on a 0 to 10 scale.

Overall, the color of the inoculated samples was significantly different from the color of the control and holder control samples. As the storage time increased, the color differences diminished, possibly due to the accumulation of bacterial metabolites based on the growth of *P. fluorescens*. The storage temperature had an effect on the sensory ratings. The sensory scores for samples inoculated with both microorganisms and stored at 11 °F were higher than the scores for the samples inoculated with both microorganisms and stored at 1 °F. This was as expected since the microbial loads were higher at higher storage temperatures.

Table 4-11 Sensory scores for whole milk associated with *P. fluorescens* and *P. conjugata* in combination study

Microorganism	Storage Time (Days)	Sensory Ratings		
		1 = No	7 =	13 =
Control	0 Avg. ^a	0.00	0.00	0.00
	0 St. dev.	0.00	0.00	0.00
	3 Avg.	0.00	0.00	0.00
	3 St. dev.	0.00	0.00	0.00
	5 Avg.	1.00	0.50	0.00
	5 St. dev.	0.74	0.48	0.29
	7 Avg.	1.00	1.00	0.75
	7 St. dev.	0.00	0.00	0.00
	10 Avg.	0.00	1.00	1.00
	10 St. dev.	0.00	0.00	0.00
<i>Pseudomonas fluorescens</i> and <i>Bacillus conjugata</i>	0 Avg.	0.00	0.00	0.00
	0 St. dev.	0.00	0.00	0.00
	3 Avg.	0.50	0.50	1.00
	3 St. dev.	0.41	0.41	0.75
	5 Avg.	0.50	1.00	1.00
	5 St. dev.	0.41	0.75	1.00
	7 Avg.	1.00	1.00	1.00
	7 St. dev.	1.00	1.00	1.00
	10 Avg.	1.00	1.00	1.00
	10 St. dev.	1.00	1.00	1.00
Hidden Control	0 Avg.	-	-	-
	0 St. dev.	-	-	-
	3 Avg.	0.00	0.00	1.00
	3 St. dev.	0.00	0.00	1.00
	5 Avg.	0.00	0.00	0.00
	5 St. dev.	0.00	0.00	1.00
	7 Avg.	0.00	0.00	0.00
	7 St. dev.	1.00	0.00	0.00
	10 Avg.	0.00	0.00	0.00
	10 St. dev.	0.00	0.00	0.00

The 0 to 13 scale was used (0 = no difference and 13 = very different)

Avg.^a Average of ten readings

St. dev. Standard deviation of ten readings

Table 4-18 Average sensory scores (intensity of whole milk inoculated with *P. fluorescens* and *B. coagulans* in combination study)

Storage Time (Days)	Sample	Sensory Scores								
		1 °C			7 °C			12 °C		
		C	PT + Bc	Bc	C	PT + Bc	Bc	C	PT + Bc	Bc
0	Avg. ^a	0	0	-	0	0	-	0	0	-
1	Avg.	0	1	1	0	1	0	0	1 ^b	1 ^b
4	Avg.	1	1	0	1 ^b	1 ^b	1 ^b	1	1 ^b	1 ^b
7	Avg.	1	1	1	1 ^b	2 ^c	1 ^b	1	1 ^b	1 ^b
10	Avg.	0 ^c	1 ^c	1 ^c	1 ^c	1 ^c	0 ^c	1	1 ^c	1 ^c

The 0 to 10 scale was used (0 = no difference and 10 = very different)

^a Avg. - Average of ten readings

C - Control samples

PT + Bc - Samples inoculated with *P. fluorescens* and *B. coagulans*

Bc - Bifidobacterium

^{b,c} Superscripts in a row within each storage time denote significant difference storage of the $p < 0.01$. Means were separated using LSD

Electronic Nose Measurements

Electronic nose sensor data showed very good classification results based on microbial counts and sensory scores for whole, reduced fat and fat-free milk inoculated with *P. fluorescens* or *B. coagulans*. Electronic nose sensor data was analyzed separately for each experiment. Representations of whole milk study for each microorganism were also combined to observe the degree of classification on pooled data. In this section, only results of the whole milk study is explained in detail, reduced fat and fat-free milk studies were explained briefly since these studies had similar trends with the whole milk study. Due to the lack of the electronic nose sensor data, it can be obtained from Dr. Murat Bulbulcu, Food Science and Human Nutrition Department at the University of Florida (Bulbulcu "Electronic nose data for").

The discriminant analysis was able to discriminate the odor changes in whole milk experiment 1 samples based on *P. fluorescens* counts with accuracy of 100% for storage temperatures of 1 °F, 7 °F, and 13 °F (Table 4-13). DFA was also capable of separating the data into different microbial loads for *E. coli* samples with correct classification rates of 100%, 100% and 91% for the storage temperatures of 1 °F, 7 °F, and 13 °F, respectively (Table 4-17). By using DFA for whole milk experiment 1 samples stored at three different temperatures, the twelve sensor outputs were reduced to two discriminant functions (Table 4-18) to calculate coordinates of points which were mapped on the two-dimensional plane. The two-dimensional plots generated from the discriminant functions showed a clear separation of the microbial counts for *P. fluorescens* and *E. coli* samples. Figure 4-8 shows results from the DFA for the whole milk experiment 1 samples inoculated with *P. fluorescens* and Figure 4-9 shows results from the DFA for the whole milk experiment 1 samples inoculated with *E. coli* samples. All samples showed perfect separation of the clusters.

The data of the whole milk experiment 1 samples at all storage temperatures were pooled together and by using DFA, the twelve sensor outputs were reduced to two discriminant functions (Table 4-19). These two functions (all $\text{temp}_{\text{max}} + \text{min}$) were used to calculate coordinates of points for data at each storage temperature which were mapped on the two-dimensional plane. For example, for *P. fluorescens* 12 sensor readings at 1 °F were taken and these values were multiplied with the specific coefficients of discriminant function 1 and function 2 and summed with the constant. Then it was plotted on the two-dimensional plot. All data were processed this way. Data from each

Table 4-17 Correct classifications were obtained from the DFA of electronic nose sensor readings compared with microbial counts of whole milk, experiment 1 and experiment 2 samples separately

Microorganism	Microbial Counts	Correct Classification Rate (%)							
		Storage Temperatures							
		Refrigerant 1				Refrigerant 2			
		1 °C	2 °C	12 °C	All Temp	1 °C	2 °C	12 °C	All Temp
Psychrotrophic Bacteria	n	25	25	25	75	25	25	25	75
	10 ²	100/00	100/00	100/00	100/0	100/00	100/00	100/00	100/0
	10 ³	100/00	100/00		66/33	100/00	100/00	—	60/00
	10 ⁴	100/00	—	—	66/33	60/00	100/00	—	70/00
	10 ⁵	—	100/00	—	66/33	100/00	—	—	100/0
	10 ⁶	100/00	100/00	100/00	33/33	—	100/00	100/00	90/00
	10 ⁷	—	100/00	100/00	70/33	—	100/00	100/00	80/00
Aerobic mesophiles	n	25	25	25	75	25	25	25	75
	10 ²	100/00	100/00	100/00	70/33	—	—	—	—
	10 ³	100/00	100/00	60/00	93/33	100/00	—	—	100/00
	10 ⁴	—	—	—	—	100/00	100/00	—	60/00
	10 ⁵	100/00	100/00	100/00	100/0	100/00	100/00	100/00	60/33
	10 ⁶	—	—	—	—	100/00	100/00	100/00	75/00
	Overall	100/00	100/00	70/00	63/33	100/00	100/00	100/00	63/33

n = number of electronic nose readings used to obtain the DFA. Numbers

Table 4.18. DPA coefficients for individual codes included in different time series models. Whole air exposures: 1 sample included with 100 subjects; 2 samples: 200 subjects; 3 samples: 300 subjects

Average Temperature (Percent)	Coefficients ($T_c = 0$ - different from sensor type)														
	T_{00}	T_{01}	T_{02}	T_{03}	T_{04}	T_{05}	T_{06}	T_{07}	T_{08}	T_{09}	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}
17°C _{max}	49.16	10.26	11.14	11.43	11.46	11.15	11.15	11.15	11.15	11.41	11.41	11.41	11.41	11.41	11.41
17°C _{min}	49.11	4.10	10.10	10.13	11.10	11.11	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
17°C _{avg}	12.71	11.20	10.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
17°C _{max}	11.00	4.00	10.00	10.11	11.10	11.11	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
17°C _{min}	11.13	4.10	11.11	11.11	11.10	11.11	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
17°C _{avg}	11.11	11.20	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
18°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
18°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
18°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
18°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
18°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
18°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
19°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
19°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
19°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
19°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
19°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
19°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
20°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
20°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
20°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
20°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
20°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
20°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
21°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
21°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
21°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
21°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
21°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
21°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
22°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
22°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
22°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
22°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
22°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
22°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
23°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
23°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
23°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
23°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
23°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
23°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
24°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
24°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
24°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
24°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
24°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
24°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
25°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
25°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
25°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
25°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
25°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
25°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
26°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
26°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
26°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
26°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
26°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
26°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
27°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
27°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
27°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
27°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
27°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
27°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
28°C _{max}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
28°C _{min}	41.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
28°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
28°C _{max}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
28°C _{min}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
28°C _{avg}	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
29°C _{max}	41.10	11.10	11.10	11.10	11.10										

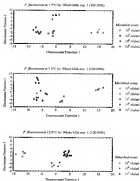


Figure 4-4 DFA of colors of whole milk experiment 1 samples inoculated with P fluorescent and stored at 1 °C, 7 °C and 13 °C based on microbial counts and electronic color readings.

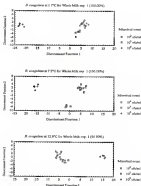


Figure 4-9 PCA of milk of whole milk experiment 1 samples separated with *B. coagulans* and stored at 1 °C, 2 °C, and 12 °C based on microbial counts and electronic nose readings

storage temperatures were plotted on separate graphs (Figures 4-10 and 4-11). By using the same discriminant functions for each storage temperature, the clusters on the graphs could be comparable with each other. Figure 4-11 shows that the clusters for microbial counts of 10^2 , 10^3 and 10^4 did not appear in the same space on the three graphs. This method could be very beneficial to the dairy industry since they can predict the microbial count of milk sample using DFA and these types of graphs, but the graphs presented here contained small numbers of samples. That is why the correct classification rate for microbial counts for all temperatures was 33% and 10.33% for *P. fluorescens* and *B. cereus*, respectively (Table 4-17). Large data sets are needed in order to get better results.

Table 4-18 contains the coefficients used to construct the discriminant functions based on microbial counts for whole milk experiment 2 samples inoculated with *P. fluorescens* or *B. cereus*. The correct classification rates for the discriminant functions of *P. fluorescens* were 94%, 100% and 100%, for storage temperatures of 1 °C, 1.2°, and 12 °C, respectively (Table 4-17). The correct classification rates for *B. cereus* were 100% for all temperatures. Figures 4-12 and 4-13 show the scatter plots obtained with the discriminant functions of milk inoculated at each storage temperature. The clusters were well defined and there was good separation between microbial count clusters. The scatter plot for *B. cereus* at 12 °C could not be drawn since only one discriminant function was generated.

When the data for experiment 1 and experiment 2 were pooled together, the discriminant function analysis calculated functions (Table 4-20) had correct classification

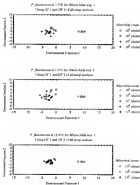


Figure 4-18 DFA of *adon* of whole body experiment 1 samples based on intertidal counts and electronic nose readings using discriminant function 1 and 2 obtained from DFA of all temperature analysis.

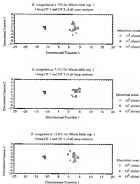


Figure 4-18 DPA of offset of white hills experiment 1 samples based on microcalorimetric events and electronic nose readings using distribution functions 1 and 2 obtained from DPA of all temperature analyses.

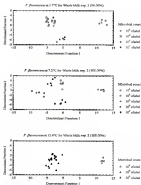


Figure 4-12. PCA of odors of whole milk experiment 1 samples inoculated with *P. fluorescens* and stored at 1 °C, 12 °C and 12 °C based on microbial counts and electronic nose readings

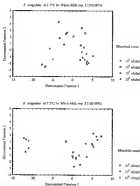


Figure 4-13 EPA of culture of whole milk experimental 2 samples inoculated with *B. conjugens* and stored at 1 °C and 7 °C based on microbial counts and electronic nose readings.

Table 2.20: DPA coefficients for mixed events recorded in detectors with error readings. Whole cell responses (1 and 2) samples correlated with T_{end} maximum fluctuations or absolute magnitude

Range Temperature (°C/min)	Coefficients(T_{end} + electron ion sensor type)													
	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}	T_{end}
T_{end} maximum fluctuations	1.75°C/min	1.80	-0.01	-1.50	11.65	-10.11	1.11	-0.19	-0.19	16.60	-12.56	-4.40	6.66	1.41
	1.75°C/min	-0.40	-0.01	0.11	0.59	-0.11	-1.14	-0.55	1.11	-11.79	10.66	1.09	-0.10	-0.10
	1.75°C/min	1.90	-0.11	0.01	1.90	-0.11	1.08	-0.10	-0.10	11.25	-0.11	11.11	0.11	1.01
	1.75°C/min	-0.40	1.11	0.11	11.01	-0.11	-1.10	11.11	-0.11	1.11	0.11	0.11	-0.11	1.01
	1.75°C/min	-1.10	0.11	0.11	11.11	11.11	1.01	0.11	1.01	-0.11	11.11	0.11	-0.11	-1.11
T_{end} absolute magnitude	1.75°C/min	1.80	0.10	-1.01	-1.10	-1.10	1.01	0.11	1.01	-0.11	0.11	11.11	-0.11	-1.11
	1.75°C/min	-0.10	1.10	-0.10	1.10	1.10	1.01	0.11	1.01	-0.11	0.11	11.11	-0.11	-1.11
	1.75°C/min	1.80	0.10	-1.01	-1.10	-1.10	1.01	0.11	1.01	-0.11	0.11	11.11	-0.11	-1.11
	1.75°C/min	-0.10	1.10	-0.10	1.10	1.10	1.01	0.11	1.01	-0.11	0.11	11.11	-0.11	-1.11
	1.75°C/min	1.80	0.10	-1.01	-1.10	-1.10	1.01	0.11	1.01	-0.11	0.11	11.11	-0.11	-1.11
Electron ion sensor	1.75°C/min	1.80	-0.11	-1.01	11.11	-1.11	-1.11	-0.11	-0.11	11.11	11.11	-0.11	-0.11	-1.11
	1.75°C/min	-0.11	1.11	-0.11	11.11	11.11	-0.11	-0.11	-0.11	-0.11	-0.11	11.11	-0.11	-1.11
	1.75°C/min	1.80	-0.11	-1.01	11.11	-1.11	-1.11	-0.11	-0.11	11.11	11.11	-0.11	-0.11	-1.11
	1.75°C/min	-0.11	1.11	-0.11	11.11	11.11	-0.11	-0.11	-0.11	-0.11	-0.11	11.11	-0.11	-1.11
	1.75°C/min	1.80	-0.11	-1.01	11.11	-1.11	-1.11	-0.11	-0.11	11.11	11.11	-0.11	-0.11	-1.11
* Sensor type = T_{end}														

rates of 90%, 98% and 99% for *P. fluorescens* at 1 °C, 7 °C, and 12 °C, respectively, and of 92%, 99%, and 100% for *B. coagulans* at 1 °C, 7 °C, and 12 °C, respectively (Table 4-21). Figures 4-14 and 4-15 show the scatter plots obtained with the discriminant functions of *P. fluorescens* and *B. coagulans* counts at three different temperatures. The clusters were separated well.

The electronic nose was able to discriminate the odor changes in reduced-fat milk samples based on the inoculated microorganisms with accuracies of 100% for storage temperatures of 1 °C, 7 °C and 12 °C (Table 4-22) except for the samples of experiment 2 inoculated with *P. fluorescens* and stored at 1 °C. The correct classification rates were 91-100% and 92-97% for samples inoculated with *P. fluorescens* experiment 1 and 2, respectively, for all temperatures and 79-87% and 90-95% for the samples of all temperatures inoculated with *B. coagulans* experiment 1 and 2, respectively.

The electronic nose was also able to discriminate the odor changes in fat-free milk samples based on the inoculated microorganisms with accuracies of 100% for storage temperatures of 1 °C, 7 °C and 12 °C (Table 4-23) except for the samples of experiment 1 inoculated with *P. fluorescens* and stored at 12 °C. The correct classification rates were 94-100% and 71-100% for samples inoculated with *P. fluorescens* experiment 1 and 2, respectively, for all temperatures and 81-100% and 94-100% for the samples of all temperatures inoculated with *B. coagulans* experiment 1 and 2, respectively.

Electronic Nose Analysis Based on Sensory Scores

The electronic nose was able to discriminate the odor changes based on sensory scores in whole milk experiment 1 and experiment 2 with high accuracies. DFA was

Table 4-21 Current classification rates obtained from the DPA of electrode type sensor readings compared with microbial counts of whole milk experiment 1 and experiment 2 samples stored aseptically

Microbial growth	Microbial Counts	Current Classification Rate (%)		
		Storage Temperature		
		1 °C	7.2 °C	22.8 °C
Pseudomonas fluorescens	n	50	50	50
	10 ²	100-100	100-100	100-100
	10 ³	94-100	100-100	-
	10 ⁴	94-100	100-100	-
	10 ⁵	100-100	100-100	-
	10 ⁶	100-100	100-100	100-100
	10 ⁷	-	100-100	100-100
	Overall	98-100	98-100	94-100
Bacillus coagulans	n	50	50	50
	10 ²	100-100	100-100	100-100
	10 ³	98-100	100-100	98-100
	10 ⁴	98-100	100-100	-
	10 ⁵	98-100	94-100	100-100
	10 ⁶	100-100	100-100	100-100
	Overall	99-100	98-100	99-100

n = number of electrode type readings used to obtain the DPA function

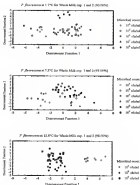


Figure 4-15 PCA of whole of whole milk experiment 1 and experiment 2 samples inoculated with *P. fluorescens* and stored at 17°, 7.5°, and 12.5°C based on microbial source and electronic noise readings

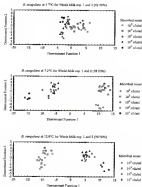


Figure 4-18 DCA of colors of whole milk experiment 1 and experiment 2 samples inoculated with *B. cereus* and stored at 17°, 27°, and 37 °C based on microbial counts and electronic nose readings

Table 4.12 Current classification rates obtained from the DPA of electronic nose sensor readings compared with microbial counts of reduced-fat milk experiments 1 and experiment 2 samples separately

Microorganisms	Microbial Counts	Current Classification Rate (%)							
		Storage Temperature							
		Experiment 1				Experiment 2			
		1 °C	7 °C	12 °C	All Temp.	1 °C	7 °C	12 °C	All Temp.
Psychrotrophic bacteria	n	25	25	25	75	25	25	25	75
	10 ²	100-00	100-00	100-00	93-3	70-00	100-00	100-00	86-66
	10 ³	100-00	-	-	99-3	100-00	100-00	-	70-00
	10 ⁴	100-00	-	-	99-3	100-00	-	-	100-0
	10 ⁵	100-00	100-00	-	98-00	100-00	-	-	89-33
	10 ⁶	-	-	-	-	-	100-00	100-00	92-00
	10 ⁷	100-00	100-00	100-00	92-00	-	100-00	-	60-00
	10 ⁸	-	100-00	100-00	98-00	-	100-00	99-00	72-20
	10 ⁹	-	-	-	-	-	-	100-00	100-0
Overall		100-00	100-00	100-00	92-00	94-00	100-00	100-00	92-47
Bacterial competitors	n	25	25	25	75	25	25	25	75
	10 ²	-	-	-	-	100-00	-	100-00	60-00
	10 ³	-	-	-	-	-	100-00	100-00	74-00
	10 ⁴	-	97-00	-	49-33	100-00	100-00	100-00	91-33
	10 ⁵	100-00	100-00	-	85-33	-	-	100-00	80-00
	10 ⁶	100-00	100-00	100-00	92-00	100-00	100-00	100-00	100-0
	10 ⁷	100-00	100-00	100-00	100-0	-	-	-	-
	10 ⁸	-	-	-	-	-	-	-	-
	Overall	100-00	100-00	100-00	79-47	100-00	100-00	100-00	82-00

n = number of electronic nose readings used to obtain the DPA functions

Table 4-10 Current classification rates obtained from the DFA of electronic nose sensor readings compared with microbial counts of bio-film with experiment 1 and experiment 2 samples (experiment 1)

Microorganism	Microbial Counts	Current Classification Rate (%)							
		Storage Temperature							
		Experiment 1				Experiment 2			
		1 °C	7 °C	10 °C	All Temp	1 °C	7 °C	10 °C	All Temp
<i>Pseudomonas fluorescens</i>	n	23	31	34	75	25	23	23	75
	10 ²	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100
	10 ³	100-100	-	-	100-100	100-100	-	-	100-100
	10 ⁴	100-100	100-100	-	100-100	-	100-100	-	100-100
	10 ⁵	100-100	100-100	100-100	100-100	100-100	100-100	-	100-100
	10 ⁶	100-100	100-100	-	100-100	-	100-100	100-100	100-100
	10 ⁷	-	100-100	100-100	100-100	-	100-100	100-100	100-100
	10 ⁸	-	-	-	-	-	-	100-100	100-100
	Overall	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100
<i>Shewanella putrefaciens</i>	n	25	25	23	75	25	23	23	75
	10 ²	-	-	-	-	100-100	-	-	100-100
	10 ³	100-100	-	-	100-100	100-100	-	-	100-100
	10 ⁴	100-100	100-100	100-100	100-100	100-100	100-100	-	100-100
	10 ⁵	-	-	100-100	100-100	-	100-100	100-100	100-100
	10 ⁶	100-100	100-100	100-100	100-100	-	100-100	-	100-100
	10 ⁷	-	-	-	-	100-100	100-100	100-100	100-100
	Overall	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100

n= number of electronic nose readings used to obtain the DFA functions

capable of separating the data into the different sensory states with correct classification rates of 100% for both microorganisms at three different temperatures for experiment 1 and 100% and 100% and 100% at 1 °C, 7.2°C, and 12.8°C, respectively for both microorganisms (Table 4-14). DFA coefficients for sensory states correlated to electronic nose sensor readings for whole milk experiment 1 inoculated with *P. fluorescens* or *B. coagulans* are given in Table 4-15 and for experiment 2 in Table 4-16. The scatter plots obtained with the discriminant functions of *P. fluorescens* for each temperature are given in Figure 4-14. The scatter plot for *B. coagulans* could not be drawn because only one discriminant function was extracted from experiment 1 data set. For experiment 2 the scatter plots are given in Figure 4-17 for *P. fluorescens* and Figure 4-18 for *B. coagulans* at three different storage temperatures. The clusters were well defined and had good separation.

When the experiment 1 and experiment 2 data were pooled together, DFA discriminated the data into the different sensory states, with correct classification rates of 88%, 100% and 100% for *P. fluorescens* and for *B. coagulans* at 1 °C, 7.2°C and 12.8°C, respectively (Table 4-17). The DFA coefficients for sensory states correlated to electronic nose sensor readings for whole milk experiment 1 together with experiment 2 samples inoculated with *P. fluorescens* or *B. coagulans* are given in Table 4-18. The scatter plots are given in Figure 4-19 and 4-20 for *P. fluorescens* and *B. coagulans*.

The electronic nose was able to discriminate the odor changes in reduced-fat milk experiment 1 and experiment 2. DFA was capable of separating the data into different sensory states with correct classification rates of 100-80% for both microorganisms at

Table 4-24 Correct classification rates obtained from the DPA of electronic nose sensor readings compared with sensory scores of whole milk, experiment 1 and experiment 2 samples separately

Information	Sensory Scores	Correct Classification Rate (%)							
		Storage Temperature							
		Experiment 1				Experiment 2			
		17°C	17°C	10-17°C	All Temp	17°C	17°C	10-17°C	All Temp
Fermentation Experiment	a	20	20	24	15	25	25	33	19
	b	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100
	c	100-100	100-100	100-100	100-100	100-100	-	-	100-100
	d	100-100	100-100	-	100-100	100-100	100-100	100-100	100-100
	e	100-100	-	-	100-100	-	100-100	-	100-100
	f	-	-	100-100	100-100	-	-	100-100	100-100
	g	-	100-100	-	100-100	-	-	100-100	100-100
	h	-	-	100-100	100-100	-	-	-	-
	i	-	-	-	-	-	-	100-100	100-100
	j	-	-	100-100	100-100	-	-	-	-
	Overall	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100
Milklet coupling	a	20	20	20	10	24	24	25	19
	b	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100
	c	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100
	d	-	-	-	-	100-100	100-100	-	100-100
	e	-	-	-	-	-	-	100-100	100-100
	f	-	-	-	-	-	-	100-100	100-100
	g	-	-	-	-	-	-	100-100	100-100
	h	-	-	-	-	-	-	100-100	100-100
	Overall	100-100	100-100	100-100	100-100	100-100	100-100	100-100	100-100

a = number of electronic nose readings used to obtain the DPA function

Table 3.11: DPA coefficients for energy scans confined to different scan vector ranges. Values with experiment 1 samples are ... , resulting with. Pseudorandom flow scans or doublet crossings

Device Temperature (T_{Device})	Coefficients ($T_{\text{sc}} = \text{different scan vector type}$)											
	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	T_{sc}	Count
Pseudorandom flow scans	1 $^{\circ}\text{C}_{\text{Device}}$	-0.19	1.14	1.15	0.47	1.11	-1.11	0.11	-0.19	1.11	11.02	2.67
	1 $^{\circ}\text{C}_{\text{Device}}$	0.19	-0.11	-0.42	1.22	1.16	-0.17	0.46	-1.12	0.11	1.14	10.18
	2 $^{\circ}\text{C}_{\text{Device}}$	-0.41	1.14	0.19	-1.12	0.19	-0.41	-1.08	0.19	-1.12	10.06	11.46
	3 $^{\circ}\text{C}_{\text{Device}}$	0.42	-1.16	1.06	1.19	0.19	-0.19	1.08	-1.12	1.06	1.14	11.46
	11 $^{\circ}\text{C}_{\text{Device}}$	11.13	-0.19	-13.06	11.42	0.17	10.14	-1.14	1.17	10.42	14.14	11.12
Pseudorandom doublet crossings	1 $^{\circ}\text{C}_{\text{Device}}$	-0.19	-0.11	-0.42	1.22	1.16	-0.17	0.46	-1.12	0.11	1.14	10.18
	1 $^{\circ}\text{C}_{\text{Device}}$	0.19	-0.11	-0.42	1.22	1.16	-0.17	0.46	-1.12	0.11	1.14	10.18
	2 $^{\circ}\text{C}_{\text{Device}}$	-0.41	1.14	0.19	-1.12	0.19	-0.41	-1.08	0.19	-1.12	10.06	11.46
	3 $^{\circ}\text{C}_{\text{Device}}$	0.42	-1.16	1.06	1.19	0.19	-0.19	1.08	-1.12	1.06	1.14	11.46
	11 $^{\circ}\text{C}_{\text{Device}}$	11.13	-0.19	-13.06	11.42	0.17	10.14	-1.14	1.17	10.42	14.14	11.12
Doublet crossings	1 $^{\circ}\text{C}_{\text{Device}}$	-0.19	1.14	1.15	0.47	1.11	-1.11	0.11	-0.19	1.11	11.02	2.67
	1 $^{\circ}\text{C}_{\text{Device}}$	0.19	-0.11	-0.42	1.22	1.16	-0.17	0.46	-1.12	0.11	1.14	10.18
	2 $^{\circ}\text{C}_{\text{Device}}$	-0.41	1.14	0.19	-1.12	0.19	-0.41	-1.08	0.19	-1.12	10.06	11.46
	3 $^{\circ}\text{C}_{\text{Device}}$	0.42	-1.16	1.06	1.19	0.19	-0.19	1.08	-1.12	1.06	1.14	11.46
	11 $^{\circ}\text{C}_{\text{Device}}$	11.13	-0.19	-13.06	11.42	0.17	10.14	-1.14	1.17	10.42	14.14	11.12
Device type = T_{Device}												

Table A-24 DNA coefficients for steady-state conditions for electrons from source readings. Whole cells represent 2 samples collected with 2 independent, identical glass fiber filters (40 µm).

Steady State Temperature (°C/°F)	Coefficients (T_e = electrons from source type)											
	T_{20}	T_{25}	T_{30}	T_{35}	T_{40}	T_{45}	T_{50}	T_{55}	T_{60}	T_{65}	T_{70}	Count
1 °C/33.8 °F	-20.48	-4.48	1.78	-42.40	48.44	-4.47	12.70	1.11	38.43	4.41	34.41	48.41
1 °C/33.8 °F	20.47	-5.11	13.48	34.75	-4.43	-4.46	4.44	-4.45	38.44	-47.44	4.41	-48.41
1 °C/33.8 °F	-49.47	1.44	1.47	34.74	11.49	4.70	1.47	12.70	-47.47	21.49	21.44	-47.44
1 °C/33.8 °F	-48.48	1.43	-43.48	3.48	40.43	1.43	13.43	38.48	11.43	3.48	38.43	13.43
1 °C/33.8 °F	-47.47	-4.47	34.74	-39.47	134.71	41.44	38.47	-41.47	134.74	37.44	41.49	11.49
10 °C/50 °F	38.47	-47.47	4.48	38.78	34.43	38.47	12.73	11.49	-44.43	3.48	-4.48	38.43
18 °C/64.4 °F	-48.47	5.13	3.43	-43.48	21.43	-4.41	13.47	4.71	38.43	4.47	38.48	11.43
all temp. °C/°F	-47.44	-4.48	-47.47	-4.44	11.47	-4.43	11.44	31.47	1.47	-47.47	12.73	12.73
1 °C/33.8 °F	47.48	1.47	1.78	38.47	-4.48	3.48	-4.48	-48.43	34.78	32.48	-47.47	31.78
1 °C/33.8 °F	-48.47	-4.48	21.48	4.43	31.48	-42.11	1.48	32.48	11.48	43.48	38.43	38.48
1 °C/33.8 °F	131.14	14.43	4.70	12.43	-47.48	12.48	-42.43	-4.48	38.48	-47.44	1.43	38.43
1 °C/33.8 °F	11.48	-4.44	1.43	-44.47	31.43	4.47	4.43	1.48	-47.47	-4.43	-44.48	-4.43
10 °C/50 °F	-41.43	1.43	1.48	13.48	11.43	-46.44	13.43	-47.47	-44.48	1.43	-44.47	-44.47
10 °C/50 °F	4.44	1.44	-47.47	1.43	-47.47	2.11	4.43	-44.48	44.48	-4.43	38.43	-4.43
all temp. °C/°F	34.44	4.48	-47.47	7.43	-44.48	7.44	4.48	-4.47	38.48	-4.43	-44.43	12.73
all temp. °C/°F	-47.44	1.48	13.43	12.48	-4.48	-4.44	-4.43	-47.47	37.44	-47.47	1.43	38.43

* Source type = T_{20}

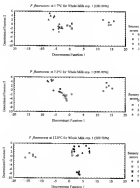


Figure 4-81: DFA of values of whale milk experiment 1 samples inoculated with $P_{\text{discriminant}}$ and stored at 1 °C, 7 °C, and 15 °C based on sensory scores and electronic nose readings.

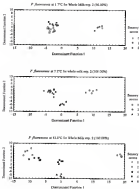


Figure 4-37 DPA of colors of whole milk experiment 2 samples inoculated with *P. fluorescens* and stored at 1 °C, 7 °C, and 13.4 °C based on sensory scores and discriminant score readings.

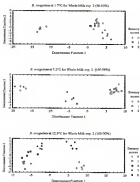


Figure 4-10 DFA of whale data with experiment 3 samples inoculated with *E. coli* *expositum* and stored at 1 °C, 7 °C, and 12 °C based on sensory status and electronic nose readings.

Table 4.22 Correct classification rates obtained from the DVA of electronic waste waste ratings compared with survey scores of waste with experience 1 and experience 2 samples pooled together

Microsegment	Survey Scores	Correct Classification Rate (%)		
		Storage Temperatures		
		1 °C	7-10°C	32 °C
Problems/Successes	n	50	50	50
	0	100.00	100.00	100.00
	1	80.00	100.00	100.00
	2	90.00	100.00	100.00
	3	60.00	100.00	-
	4	-	-	100.00
	5	-	100.00	100.00
	7	-	-	100.00
	8	-	-	100.00
	9	-	-	100.00
	Overall	88.00	100.00	100.00
Recalls/compilers	n	50	50	50
	0	60.00	100.00	100.00
	1	94.67	100.00	100.00
	2	60.00	100.00	-
	4	-	-	100.00
	7	-	-	100.00
	8	-	-	100.00
	Overall	83.33	100.00	100.00

n= number of electronic waste ratings used to derive the DVA, Success

It's common for many men enrolled in domestic violence programs to be charged with domestic violence in their civilian lives. This is because they are charged with domestic violence in their civilian lives.

[illegible]

10

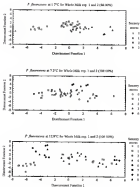


Figure 4-28 *P*-fluorescence of whole milk, experiment 1 and experiment 2 samples irradiated with *P*-fluorescence and stored at 1 °C, 7 °C, and 12 °C based on sensory notes and electronic nose readings

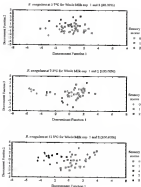


Figure 4-10 DFA of whale milk samples with discriminant 1 and discriminant 2 samples associated with *B. megaptera* and stored at 17°C, 17°C, and 12-16°C based on sensory scores and electronic nose readings.

three different temperatures for reduced-fat experiment 1 and 00%, 100% and 100% for experiment 2 samples inoculated with *P. fluorescens* stored at 1 °F, 7.2°, and 12 °F, respectively (Table 4-29). The correct classification rates for fat-free milk experiment 1 and experiment 2 samples are given in Table 4-30. They all had high classification rates.

Accelerated Study for All Types of Milk

In the accelerated study, the electronic nose sensor readings of the accelerated study for each type and experiment of milk were subtracted from the sensor readings of the previous day's experiment. The difference between twelve sensor readings of *P. fluorescens* at consecutive days was analyzed using Principal Component Analysis (PCA). PCA was used to observe if the data can be separated into different groups. Results showed that factor one had very high separation compared to factor two (Table 4-31), meaning that PCA explains most of the data as one component and the results were called BPC1 while the PCA results for all the previous days data were named as BPC2.

Remaining shelf life was found from the curves of the samples inoculated with *P. fluorescens* and stored at 1 °F (Figures 4-1 to 4-3). 7.5 Log₁₀ shelf life was considered as the end of shelf life. When a line parallel to the mean passing through the last stored the 1 °F microbial growth curve, the storage time was recorded as the shelf life for that milk. At each day of the accelerated study, that day was subtracted from shelf life of each milk.

Figures 4-21 to 4-23 show the change of remaining shelf life with respect to BPC1 and BPC2 for all milk types and experiments. As seen in these figures, experiments 1 and 2 did not follow similar trends. Therefore, it does not seem to be possible to use this data to predict the shelf life. However, if these experiments were performed with larger

Table 9-29 Correct classification rates obtained from the DFA of electronic nose sensor readings compared with sensory scores of individual milk samples (Experiment 1 and experiment 2 samples separately)

Sensory categories	Sensory Scores	Correct Classification Rate (%)							
		Storage Temperatures							
		Experiment 1				Experiment 2			
		1 °C	3 °C	11 °C	All Temp	1 °C	3 °C	12 °C	All Temp
Fermentation / Sourness	n	25	25	25	75	25	25	25	75
	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	1	100.0	-	-	100.0	90.0	-	100.0	93.3
	2	100.0	100.0	-	73.3	100.0	100.0	-	73.3
	3	100.0	100.0	-	-	-	100.0	-	40.0
	4	-	-	-	-	-	-	100.0	100.0
	5	-	100.0	-	66.6	-	-	-	-
	6	-	-	100.0	100.0	-	-	-	-
	7	-	-	100.0	70.0	-	100.0	100.0	93.3
	8	-	-	-	-	-	-	100.0	100.0
	Overall	100.0	100.0	100.0	74.4	98.0	100.0	100.0	94.0
Bacterial spoilage	n	25	25	25	75	25	25	25	75
	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	1	100.0	100.0	100.0	40.0	100.0	100.0	100.0	80.0
	2	100.0	100.0	100.0	43.3	100.0	80.0	100.0	76.6
	3	-	-	-	-	-	100.0	-	40.0
	4	-	-	-	-	-	-	100.0	40.0
	Overall	100.0	100.0	100.0	74.4	100.0	94.0	100.0	81.3

n = number of electronic nose readings used to obtain the DFA functions

Table 4-28 Correct classification rates obtained from the DFA of electrocardiogram sensor readings compared with sensory scores of far-field milk experiment 1 and experiment 2 periods separately

Microsensor type	Sensory Scores	Correct Classification Rate (%)							
		Storage Temperature							
		Experiment 1				Experiment 2			
		1 °C	1 °C	12 °C	all Temp	1 °C	7 °C	12 °C	all Temp
Paraflex [®] Microsensor	n	25	25	25	75	24	24	25	73
	0	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100
	1	-	100/100	-	40/100	100/100	100/100	-	100/100
	2	100/100	-	100/100	33/33	100/100	100/100	-	100/100
	3	100/100	-	-	100/100	-	-	-	-
	4	-	100/100	-	80/100	100/100	100/100	100/100	100/100
	5	-	100/100	-	100/100	-	-	-	-
	7	-	-	100/100	100/100	-	100/100	-	100/100
	8	-	-	-	-	-	-	100/100	100/100
	9	-	-	100/100	100/100	-	-	100/100	100/100
	10	-	-	100/100	100/100	-	-	100/100	100/100
	Overall	100/100	100/100	100/100	78.67	100/100	100/100	100/100	100/100
Bioflex [®] microsensor	n	25	25	25	75	24	24	25	73
	0	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100
	1	100/100	100/100	100/100	100/100	-	-	-	-
	2	100/100	-	-	100/100	100/100	100/100	100/100	100/100
	3	-	100/100	-	100/100	-	100/100	100/100	100/100
	4	-	-	-	-	-	-	100/100	100/100
	5	-	-	100/100	100/100	-	-	-	-
	8	-	-	100/100	100/100	-	-	-	-
	Overall	100/100	100/100	100/100	93.33	100/100	100/100	100/100	100/100

n = number of electrocardiogram readings used to obtain the DFA, fractions

Table 4-31 Eigenvalues of the principal component factors obtained by PCA for data sets of each type and exposure of milk

Type/Exposure	PCA	Assessment study samples accounted with P_1 (percentage) and scored at 1. PC
Whole Milk / Exp. 1	BPC 1	13.39
	BPC 2	1.66
	BPC 3	0.43
	BPC 4	0.29
Whole Milk / Exp. 2	BPC 1	8.73
	BPC 2	2.58
	BPC 3	0.26
	BPC 4	0.03
Reduced-fat Milk / Exp. 1	BPC 1	11.06
	BPC 2	0.68
	BPC 3	10.58
	BPC 4	0.07
Reduced-fat Milk / Exp. 2	BPC 1	11.22
	BPC 2	0.86
	BPC 3	10.37
	BPC 4	1.08
Fat-free Milk / Exp. 1	BPC 1	7.07
	BPC 2	2.41
	BPC 3	2.34
	BPC 4	0.34
Fat-free Milk / Exp. 2	BPC 1	13.68
	BPC 2	2.68
	BPC 3	10.81
	BPC 4	0.03

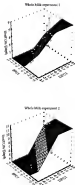


Figure 4-25. Change of shelf-life with respect to DPC (I) and BPC (I) for whole milk inoculated with *P. fluorescens* in accelerated study

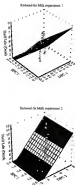


Figure 4-23. Change of shelf life with respect to DPC 1 and BPC 1 for reduced-fat milk inoculated with *F. florissimum* in accelerated study

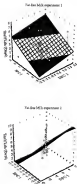


Figure 4-23 Change of shell life with respect to DPC-1 and EPC-1 for flat-skin cells inoculated with *P. fluorescens* in accelerated study

number of samples and with some initial microbial loads, a predictive model for shelf life of milk could be developed.

Concentration Study

When *P. fluorescens* and *B. coagulans* were inoculated to the same whole milk sample, the color could not be correlated with microbial counts of each microorganism since they produced complex mixtures of colors which were difficult to compare with single specific colors produced by each microorganism. However, the electronic nose was able to discriminate the color changes in milk samples inoculated with both microorganisms with high accuracy. The classification rates based on sensory scores for inoculated samples at each storage temperature were 100% (Table 4-12). The DFA coefficients for sensory scores correlated to electronic nose sensor readings for whole milk samples inoculated with *P. fluorescens* and *B. coagulans* are given in Table 4-13. The clusters for each sensory score were defined well. The scatter plots are given in Figures 4-14 for sensory scores for both microorganisms. The predictive model for determining sensory scores of milk inoculated with *P. fluorescens* and *B. coagulans* was developed utilizing this model sensory prediction of an unknown sample could be predicted. Even though the predictor was not used in this research, it is thought that a better predictor model could have been developed and high prediction percentages could have been obtained if large number of samples were used.

Table 4-32 Current classification rates obtained from the DFA of electronic nose sensor readings compared with sensory scores of whole milk samples inoculated with *E. faecium* and *A. proteolyticus*

Microorganisms	Sensory Scores	Current Classification Rate (%)		
		Storage Temperature		
		17°C	22°C	25.0°C
<i>Enterobacteriaceae</i> & <i>Acetivibrio</i> spp.	0	25	25	25
	1	100/00	100/00	100/00
	2	100/00	100/00	100/00
	3	100/00	-	-
	4	-	100/00	-
	5	-	100/00	100/00
	6	-	-	100/00
	7	-	-	100/00
	Overall	100/00	100/00	100/00

n = number of electronic nose readings used to obtain the DFA functions

Table 4-33 MEA coefficients for anemity sensors, correlated to chlorine near sensor readings. (Note: all samples measured) with *Pseudomonas* (chlorine) and *Shewanella* (negative)

Storage Temperature (°C/°F)	Coefficients (CL ₂ = chlorine near sensor type)													
	T ₀₁		T ₀₂		T ₀₃		T ₀₄		T ₀₅		T ₀₆		T ₀₇	
	T ₀₁	T ₀₂	T ₀₃	T ₀₄	T ₀₅	T ₀₆	T ₀₇	T ₀₈	T ₀₉	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄
1.1°C/34.0°F	-0.032	1.00	-0.41	-1.33	-1.33	0.00	0.00	0.00	-0.13	1.00	-0.13	-0.13	-0.13	-0.13
1.1°C/34.0°F	-0.032	-0.00	-0.33	-0.33	-0.33	0.00	0.00	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13
1.1°C/34.0°F	0.00	1.00	-0.33	-0.33	-0.33	-0.13	0.00	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13
1.1°C/34.0°F	0.00	-0.00	-0.33	-0.33	-0.33	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13
1.1°C/34.0°F	-0.13	0.00	0.00	-0.33	-0.33	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13
1.1°C/34.0°F	0.00	-0.00	-0.33	-0.33	-0.33	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13	-0.13

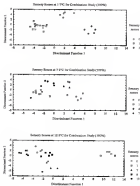


Figure 4-24 DSA of mine of whale with insulated with both microcapsules and mineral at 11°C, 13°C, and 15°C based on density matrix and electronic micro-imagery

CHAPTER 3 CONCLUSIONS AND RECOMMENDATIONS

The study concluded that the odor change of milk samples associated with *Pseudomonas fluorescens* and/or *Acidithiobacillus* can be measured objectively and rapidly with the electronic nose technology, giving results similar to those obtained from sensory panels. A predictive model for sensory evaluation of milk was developed. This research also showed that microbial counts of pure cultures could be correlated with the electronic nose sensor readings. A predictive model for determining microbial load of milk was also developed. Independent validation for these predictive models was not done since the experiments 1 and 2 performed using the same type of milk were not the same due to different initial inoculation levels.

The results obtained from the uncalibrated study showed that with this data set and with the approach described, it was not possible to predict shelf life of a milk sample by electronic nose sensors. The odor produced by the organisms due to microbial growth could be different because the initial microbial loads used in this study were not the same.

Further work is needed to develop larger data sets by studying many experiments that could be used to predict the sensory scores and microbial counts of milk. The initial loads for the samples need to be the same or very close. When the initial loads for both experiments will be the same, they can be comparable.

In this study DPA was used as the pattern-recognition technique, however artificial neural networks could be used. This should allow researchers to take into account individual level of angle or combinations of measurements at the time of analysis. This certainly the development of better predictive models for value of milk due to growth of osteoporosis.

APPENDIX A
DATA FOR MICROBIAL ANALYSIS

Table A-1 *Microbial load of whole milk inoculated with *Pseudomonas fluorescens**

Storage Time (Days)	Sample	Microbial Load (log ₁₀ cfu/ml of milk)					
		Experiment 1			Experiment 2		
		(Storage Temperature)			(Storage Temperature)		
		1.0°C	1.0°C	12.0°C	1.0°C	2.0°C	12.0°C
0	1	4.30	4.30	4.30	3.68	3.68	3.68
	2	4.38	4.38	4.38	3.50	3.50	3.50
	Avg. ^a	4.35	4.35	4.35	3.59	3.59	3.59
3	1	4.40	4.44	3.50	3.30	3.84	3.60
	2	4.40	4.40	3.50	3.38	3.12	3.30
	Avg.	4.37	4.34	3.50	3.34	3.48	3.45
6	1	5.42	7.28	8.88	4.78	6.10	8.10
	2	6.44	7.28	8.88	4.80	6.10	8.88
	Avg.	6.43	7.28	8.93	4.85	6.10	8.99
9	1	6.30	7.60	9.06	4.40	7.00	8.90
	2	6.40	7.60	9.10	4.40	7.00	8.90
	Avg.	6.40	7.60	9.09	4.40	7.00	8.90
12	1	8.30	9.84	9.24	6.74	8.76	8.24
	2	8.30	9.84	9.24	6.74	8.76	8.24
	Avg.	8.30	9.84	9.24	6.74	8.76	8.24

Avg.^a Average of two readings

Table A-2 Microbial load of whole milk inoculated with bacterial consortium

Storage Time (Days)	Sample	Microbial Load					
		[Log ₁₀ count of milk]					
		Experiment 1			Experiment 2		
		(Storage Temperature)			(Storage Temperature)		
		1.0°C	3.0°C	12.0°C	1.0°C	3.0°C	12.0°C
0	1	4.55	4.03	4.58	5.81	4.52	5.81
	2	4.58	4.58	4.58	5.85	5.85	5.85
	Avg. ^a	4.54	4.55	4.55	5.85	5.85	5.85
3	1	3.35	3.33	3.45	4.85	4.43	4.78
	2	3.11	3.41	3.95	4.88	4.43	4.88
	Avg.	3.35	3.42	3.74	4.86	4.48	4.81
6	1	3.22	3.43	3.11	4.33	3.15	3.21
	2	3.43	3.35	3.35	4.58	3.48	3.35
	Avg.	3.42	3.41	3.33	4.56	3.34	3.29
9	1	3.65	3.13	3.55	5.35	4.44	3.28
	2	3.64	3.45	3.33	5.48	4.55	4.58
	Avg.	3.65	3.43	3.33	5.45	4.55	4.58
12	1	3.64	3.65	3.65	5.38	4.45	4.75
	2	3.68	3.35	3.35	5.48	4.42	4.88
	Avg.	3.64	3.48	3.48	5.45	4.43	4.81

Avg.^a Average of two readings

Table A-3 **Morshel loss of reduced-fat milk inoculated with *Penicillium* *Roqueforti***

Storage Time (Days)	Sample	Morshel Loss					
		[mg. oil/l. of milk]					
		Experiment 1			Experiment 2		
		(Storage Temperature)			(Storage Temperature)		
		1.7°C	7.7°C	15.0°C	1.7°C	7.7°C	15.0°C
0	1	3.35	3.35	3.31	3.40	3.40	3.43
	2	3.35	3.35	3.35/4	3.40	3.40	3.43
	Avg. ^a	3.35	3.35	3.33/4	3.40	3.40	3.43
3	1	3.88	3.83	3.43	3.48	3.38	3.43
	2	3.87	3.75	3.40	3.48	3.38	3.43
	Avg.	3.88	3.79	3.43	3.48	3.38	3.43
6	1	3.34	3.25	4.30	3.40	3.30	4.31
	2	3.35	3.25	4.31	3.44	3.30	4.31
	Avg.	3.35	3.25	4.30	3.42	3.30	4.31
9	1	4.45	4.50	4.30	5.18	4.30	4.30
	2	4.48	4.50	4.30	5.25	4.40	4.31
	Avg.	4.46	4.50	4.30	5.18	4.35	4.30
30	1	5.30	4.60	4.40	4.18	5.00	4.30
	2	5.38	4.61	4.40	4.45	5.00	4.31
	Avg.	5.34	4.61	4.40	4.30	5.00	4.30

Avg.^a Average of two readings

Table A-4 Microbial load of reduced-fat milk inoculated with *Bacillus cereus*

Storage Time (Days)	Sample	Microbial Load					
		log ₁₀ (count of cells)					
		Refrigerator 1			Refrigerator 2		
		(Storage Temperature)			(Storage Temperature)		
		1.7°C	2.2°C	10.0°C	1.7°C	2.2°C	10.0°C
0	1	5.91	5.57	5.91	5.11	5.71	5.11
	2	5.45	5.55	5.83	5.15	5.75	5.15
	Avg. ^a	5.67	5.57	5.87	5.11	5.71	5.11
3	1	5.26	4.94	5.43	5.04	5.43	5.43
	2	5.56	4.94	5.94	4.95	5.46	5.95
	Avg.	5.36	4.94	5.69	5.04	5.45	5.69
6	1	4.45	4.45	5.14	3.85	5.05	5.05
	2	4.55	4.44	5.15	3.85	5.05	5.05
	Avg.	4.45	4.44	5.15	3.85	5.05	5.05
9	1	3.77	3.87	4.69	3.66	5.08	5.05
	2	3.94	3.99	4.94	3.66	5.08	5.05
	Avg.	3.87	3.93	4.82	3.66	5.08	5.05
12	1	3.72	3.65	4.76	3.65	5.05	5.05
	2	3.76	3.65	4.79	3.65	5.05	5.05
	Avg.	3.74	3.65	4.77	3.65	5.05	5.05

Avg.^a Average of two readings

Table A.8. Mortality level of 30-day cells inoculated with *Trachinotus* (Survivors)

Storage Time (Days)	Sample	Mortality Level					
		(log ₁₀ count of cells)					
		Repayment 1			Repayment 2		
		(Storage Temperature)			(Storage Temperature)		
		1.7°C	1.7°C	12.8°C	1.7°C	1.7°C	12.8°C
0	1	4.66	4.64	4.64	4.28	4.28	4.28
	2	3.96	3.95	3.95	4.36	4.36	4.36
	Avg. ^a	3.99	3.95	3.99	4.32	4.32	4.32
3	1	5.21	4.68	7.00	5.84	5.20	7.54
	2	5.20	4.74	7.00	5.84	4.85	7.54
	Avg.	5.21	4.71	7.00	5.84	5.12	7.54
6	1	4.36	5.28	6.58	4.56	4.91	6.15
	2	4.34	5.48	6.55	4.63	4.90	6.04
	Avg.	4.34	5.43	6.57	4.71	4.91	6.11
9	1	4.72	4.64	6.11	4.20	4.68	6.20
	2	4.72	5.68	6.11	4.28	4.71	6.20
	Avg.	4.72	5.16	6.11	4.24	4.69	6.20
10	1	5.41	4.21	6.55	5.56	4.85	6.58
	2	5.44	4.57	6.58	5.56	4.80	6.62
	Avg.	5.44	4.51	6.57	5.56	4.83	6.60

Avg.^a Average of two readings

Table A-4. Monitored load of (a) free milk measured with double receptors

Storage Time (Days)	Sample	Monitored Load (kg, total of milk)					
		(kg, total of milk)					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.2°C	7.2°C	12.0°C	1.2°C	7.2°C	12.0°C
0	1	5.33	5.33	5.30	5.00	5.00	5.00
	2	5.33	5.33	5.30	5.00	5.00	5.00
	Avg. ^a	5.33	5.33	5.30	5.00	5.00	5.00
1	1	5.05	5.08	5.08	5.04	4.91	4.91
	2	5.08	5.08	5.08	5.03	4.93	4.90
	Avg.	5.06	5.08	5.08	5.04	4.92	4.90
3	1	5.05	5.05	5.00	5.00	5.00	4.94
	2	5.00	5.04	5.04	5.00	5.00	4.90
	Avg.	5.02	5.05	5.02	5.00	5.00	4.92
7	1	5.00	5.00	5.00	5.00	5.00	5.00
	2	5.00	5.00	5.00	5.00	5.00	4.98
	Avg.	5.00	5.00	5.00	5.00	5.00	4.99
10	1	5.00	5.00	5.00	5.00	5.00	5.00
	2	5.00	5.00	5.00	5.00	5.00	5.00
	Avg.	5.00	5.00	5.00	5.00	5.00	5.00

Avg.^a Average of two readings

Table 4.7 Microbial load of whole milk inoculated with *Pseudomonas fluorescens* and *Bacillus coagulans*

Storage Time (Days)	Sample	Microbial Load					
		Org./ml of milk					
		<i>Pseudomonas fluorescens</i>			<i>Bacillus coagulans</i>		
		Storage Temperature			Storage Temperature		
		4 °C	12 °C	18 °C	4 °C	12 °C	18 °C
0	1	1.64	1.84	2.84	5.51	5.57	5.57
	2	1.78	1.78	2.79	5.82	5.82	5.82
	Avg. ^a	1.71	1.81	2.82	5.66	5.69	5.69
1	1	2.95	3.46	7.34	4.78	5.11	5.45
	2	3.18	3.40	7.34	4.97	5.30	5.58
	Avg.	3.06	3.43	7.34	4.88	5.21	5.52
2	1	2.22	4.28	8.44	4.52	5.11	5.28
	2	2.48	4.89	8.44	4.78	5.34	5.52
	Avg.	2.35	4.57	8.44	4.64	5.24	5.40
7	1	3.85	4.28	8.70	4.94	4.94	4.94
	2	3.90	4.46	8.60	4.90	4.94	4.94
	Avg.	3.88	4.37	8.65	4.94	4.94	4.94
10	1	3.78	4.87	8.36	5.75	4.84	4.97
	2	3.40	4.95	8.22	5.80	4.84	4.98
	Avg.	3.59	4.91	8.29	5.78	4.84	4.98

Avg.^a Average of two readings

Table A-8 Microbial load of whole milk inoculated with *Pseudomonas fluorescens* or *Escherichia coli* in accelerated study

Analysis Time (Days)	Sample	Microbial Load (cells/ml of milk)			
		Experiment 1		Experiment 2	
		<i>P. fluorescens</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>E. coli</i>
1	1	4.31	4.34	4.50	4.50
	2	4.31	4.34	4.50	4.50
	Avg. ^a	4.31	4.35	4.50	4.50
4	1	4.40	4.48	4.69	4.70
	2	4.35	4.48	4.75	4.76
	Avg.	4.34	4.48	4.72	4.73
6	1	4.87	5.10	5.66	5.66
	2	4.96	5.10	5.75	5.66
	Avg.	4.91	5.10	5.70	5.66
8	1	5.04	5.48	6.34	6.40
	2	5.60	5.76	6.56	6.54
	Avg.	5.34	5.70	6.45	6.47

Avg.^a Average of two readings

Table A-9 Microbial load of reduced-fat milk inoculated with *Pyrococcus* (Experiment 1) and *Acidithiobacillus* (Experiment 2) in accelerated study

Analysis Time (Days)	Sample	Microbial Load (log ₁₀ cfu/ml of milk)			
		Experiment 1 (Inoculated Microorganisms)		Experiment 2 (Inoculated Microorganisms)	
		<i>P. furiosus</i>	<i>A. cupressus</i>	<i>P. furiosus</i>	<i>A. cupressus</i>
1	1	3.81	3.64	3.92	3.68
	2	3.86	3.65	3.98	3.73
	Avg. ^a	3.84	3.64	3.95	3.71
4	1	4.74	5.81	4.55	5.11
	2	4.85	5.91	4.58	5.18
	Avg.	4.79	5.87	4.57	5.15
6	1	5.95	5.87	5.38	4.93
	2	5.85	5.94	5.48	4.99
	Avg.	5.90	5.90	5.43	4.96
8	1	6.04	5.95	5.75	4.91
	2	5.95	5.94	5.65	5.05
	Avg.	5.99	5.94	5.70	4.98

Avg.^a Average of two readings

Table A-18 Microbial load of the feed milk inoculated with *Paratuberculosis* bacterium at *Brucella* complex in accelerated study

Analysis Time (Days)	Sample	Microbial Load (log ₁₀ count of milk)			
		Experiment 1		Experiment 2	
		(Inoculated Microorganisms)		(Inoculated Microorganisms)	
		<i>C. Brucellensis</i>	<i>B. abortus</i>	<i>C. Brucellensis</i>	<i>B. abortus</i>
1	1	4.45	7.79	8.41	4.88
	2	8.18	7.79	4.45	4.76
	Avg. ^a	6.32	7.79	6.43	4.82
4	1	4.45	7.45	8.41	5.00
	2	4.45	7.45	8.41	5.00
	Avg.	4.45	7.45	8.41	5.00
6	1	9.04	7.45	8.38	5.00
	2	9.04	7.45	8.41	5.00
	Avg.	9.04	7.45	8.40	5.00
8	1	7.79	4.45	8.40	5.00
	2	8.18	4.45	8.38	5.00
	Avg.	8.00	4.45	8.39	5.00

Avg.^a Average of two readings

Table A-11 Microbial load of whole milk inoculated with *Pseudomonas fluorescens* and *Bacillus cereus* in commercial milk

Storage Time (Days)	Sample	Microbial Load (log ₁₀ count of ml)	
		Original count of milk	
		<i>Pseudomonas fluorescens</i>	<i>Bacillus cereus</i>
1	1	8.38	8.62
	2	8.38	8.75
	Avg. ^a	8.38	8.69
4	1	7.79	8.33
	2	8.11	8.83
	Avg.	7.95	8.58
8	1	8.32	8.64
	2	8.35	8.68
	Avg.	8.34	8.66
11	1	8.49	8.85
	2	8.63	8.88
	Avg.	8.56	8.87

^aAvg. Average of test readings

**APPENDIX B
DATA FOR pH**

Table B-1 pH of whole milk without inoculated with mesophilic bacteria (continued)

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.7°C	3.3°C	10.0°C	1.7°C	3.3°C	10.0°C
0	1	6.69	6.69	6.69	6.76	6.76	6.76
	2	6.77	6.77	6.77	6.76	6.76	6.76
	Avg.	6.73	6.73	6.73	6.76	6.76	6.76
	St. dev.	0.04	0.04	0.04	0.00	0.00	0.00
Day 3	1	6.65	6.62	6.62	6.71	6.71	6.71
	2	6.67	6.64	6.64	6.73	6.74	6.73
	Avg.	6.66	6.63	6.63	6.72	6.73	6.72
	St. dev.	0.01	0.01	0.00	0.01	0.01	0.01
Day 5	1	6.61	6.61	6.74	6.72	6.73	6.74
	2	6.66	6.65	6.74	6.67	6.71	6.73
	Avg.	6.63	6.63	6.74	6.70	6.72	6.73
	St. dev.	0.03	0.02	0.01	0.07	0.01	0.01
Day 7	1	6.61	6.71	6.80	6.74	6.74	6.74
	2	6.73	6.79	6.81	6.76	6.75	6.75
	Avg.	6.67	6.75	6.81	6.75	6.75	6.75
	St. dev.	0.06	0.04	0.01	0.01	0.00	0.00
Day 10	1	6.74	6.75	6.78	6.75	6.70	6.73
	2	6.74	6.77	6.79	6.74	6.73	6.74
	Avg.	6.74	6.76	6.78	6.74	6.71	6.73
	St. dev.	0.00	0.01	0.01	0.01	0.01	0.01

*Avg. = Average of two readings

St. dev. = Standard deviation of two readings

Table B-2 pH of whole milk inoculated with *Penicillium Roqueforti*

Storage Time (Days)	Sample	25°					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.75%	1.75%	15.62%	1.75%	1.75%	15.62%
Day 0	1	6.71	6.71	6.71	6.71	6.72	6.72
	2	6.71	6.71	6.71	6.68	6.80	6.80
	Avg.	6.71	6.71	6.71	6.70	6.76	6.76
	St. dev.	0.01	0.00	0.00	0.00	0.06	0.00
Day 3	1	6.64	6.63	6.64	6.71	6.69	6.71
	2	6.63	6.71	6.63	6.71	6.70	6.72
	Avg.	6.64	6.68	6.63	6.71	6.69	6.72
	St. dev.	0.11	0.09	0.00	0.01	0.11	0.01
Day 6	1	6.64	6.70	6.63	6.70	6.70	6.67
	2	6.60	6.62	6.66	6.67	6.7	6.68
	Avg.	6.64	6.66	6.65	6.69	6.70	6.68
	St. dev.	0.01	0.01	0.00	0.00	0.00	0.01
Day 7	1	6.71	6.70	6.64	6.70	6.64	6.63
	2	6.71	6.70	6.60	6.70	6.65	6.61
	Avg.	6.71	6.70	6.62	6.70	6.65	6.62
	St. dev.	0.00	0.00	0.00	0.01	0.00	0.01
Day 10	1	6.70	6.63	6.54	6.71	6.60	6.66
	2	6.70	6.58	6.50	6.71	6.74	6.68
	Avg.	6.70	6.61	6.52	6.71	6.67	6.67
	St. dev.	0.01	0.01	0.00	0.01	0.06	0.01

Avg. = Average of two readings

St. dev. = Standard deviation of two readings

Table B-3 *gilt of whole milk inoculated with *Escherichia coli* O157*

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.7°C	3.3°C	13.3°C	1.7°C	3.3°C	13.3°C
Day 0	1	6.7	6.7	6.7	6.75	6.75	6.75
	2	6.75	6.75	6.75	6.74	6.74	6.74
	Avg. ^a	6.73	6.73	6.73	6.74	6.74	6.74
	St. dev.	0.01	0.01	0.01	0.01	0.01	0.01
Day 3	1	6.81	6.87	6.87	6.75	6.77	6.75
	2	6.83	6.85	6.86	6.77	6.77	6.78
	Avg.	6.84	6.86	6.87	6.76	6.77	6.76
	St. dev.	0.04	0.01	0.01	0.01	0.01	0.02
Day 5	1	6.76	6.83	6.76	6.75	6.76	6.76
	2	6.84	6.81	6.76	6.85	6.76	6.82
	Avg.	6.80	6.82	6.76	6.77	6.76	6.83
	St. dev.	0.04	0.05	0.06	0.05	0.06	0.04
Day 7	1	6.71	6.79	6.79	6.85	6.75	6.85
	2	6.73	6.81	6.80	6.85	6.76	6.83
	Avg.	6.72	6.80	6.80	6.85	6.75	6.84
	St. dev.	0.03	0.01	0.02	0.05	0.05	0.01
Day 10	1	6.74	6.80	6.79	6.73	6.73	6.86
	2	6.75	6.78	6.79	6.73	6.75	6.81
	Avg.	6.75	6.79	6.79	6.73	6.74	6.83
	St. dev.	0.03	0.01	0.01	0.05	0.05	0.02

^a Avg. = Average of two readings

St. dev. = Standard deviation of two readings

Table 3-4 pH of reduced fat milk without inoculated with microorganisms (Continued)

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.1°C	1.1°C	12.1°C	1.1°C	1.1°C	12.1°C
0	1	6.75	6.75	6.75	6.78	6.78	6.78
	2	6.75	6.75	6.75	6.78	6.78	6.78
	Avg. ^a	6.75	6.75	6.75	6.78	6.78	6.78
	St. dev.	0.00	0.00	0.00	0.01	0.01	0.01
3	1	6.68	6.70	6.70	6.75	6.74	6.75
	2	6.70	6.71	6.71	6.78	6.78	6.78
	Avg.	6.70	6.71	6.71	6.77	6.77	6.77
	St. dev.	0.01	0.01	0.01	0.01	0.01	0.01
6	1	6.74	6.75	6.75	6.78	6.78	6.78
	2	6.76	6.76	6.76	6.77	6.77	6.78
	Avg.	6.75	6.76	6.76	6.78	6.78	6.78
	St. dev.	0.01	0.00	0.01	0.01	0.01	0.01
9	1	6.75	6.75	6.75	6.77	6.78	6.78
	2	6.75	6.75	6.69	6.78	6.78	6.74
	Avg.	6.75	6.75	6.72	6.78	6.78	6.76
	St. dev.	0.00	0.00	0.03	0.01	0.01	0.01
12	1	6.75	6.75	6.76	6.80	6.80	6.77
	2	6.75	6.75	6.76	6.80	6.80	6.76
	Avg.	6.75	6.75	6.76	6.80	6.80	6.77
	St. dev.	0.00	0.01	0.00	0.01	0.01	0.01

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Table 8-3 pH of reduced fat milk associated with *Campylobacter* fermentation

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		17°C	22°C	24.5°C	17°C	22°C	24.5°C
Day 0	1	6.87	6.87	6.87	6.78	6.75	6.73
	2	6.76	6.76	6.76	6.73	6.73	6.73
	Avg.	6.76	6.76	6.76	6.73	6.74	6.73
	St. dev.	0.13	0.13	0.13	0.06	0.06	0.06
Day 1	1	6.73	6.64	6.63	6.73	6.75	6.63
	2	6.68	6.57	6.63	6.75	6.77	6.73
	Avg.	6.70	6.70	6.63	6.74	6.76	6.68
	St. dev.	0.05	0.10	0.01	0.06	0.06	0.07
Day 3	1	6.75	6.63	6.54	6.77	6.7	6.63
	2	6.75	6.65	6.53	6.77	6.73	6.66
	Avg.	6.75	6.64	6.53	6.77	6.72	6.65
	St. dev.	0.01	0.05	0.01	0.06	0.03	0.04
Day 7	1	6.67	6.68	6.57	6.74	6.76	6.54
	2	6.68	6.64	6.48	6.74	6.75	6.53
	Avg.	6.68	6.66	6.53	6.74	6.75	6.54
	St. dev.	0.01	0.03	0.05	0.06	0.03	0.04
Day 10	1	6.55	6.67	6.76	6.64	6.71	6.73
	2	6.55	6.74	6.68	6.67	6.70	6.70
	Avg.	6.55	6.70	6.72	6.67	6.71	6.72
	St. dev.	0.00	0.09	0.01	0.03	0.03	0.01

Avg. = Average of two readings

St. dev. = Standard deviation of two readings

Table B-4. pH of reduced-fat milk measured with Hachette electrodes

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.7°C	1.9°C	12.8°C	1.7°C	1.9°C	12.8°C
0	1	6.74	6.74	6.74	6.75	6.75	6.75
	2	6.75	6.75	6.75	6.75	6.75	6.75
	Avg. ^a	6.75	6.75	6.75	6.75	6.75	6.75
	St. dev. ^b	0.01	0.01	0.04	0.00	0.00	0.00
1	1	6.73	6.70	6.73	6.70	6.73	6.73
	2	6.86	6.77	6.75	6.74	6.75	6.75
	Avg.	6.80	6.73	6.75	6.72	6.74	6.74
	St. dev.	0.07	0.03	0.01	0.00	0.04	0.01
3	1	6.75	6.73	6.7	6.73	6.73	6.73
	2	6.73	6.73	6.78	6.76	6.75	6.75
	Avg.	6.74	6.73	6.74	6.75	6.74	6.74
	St. dev.	0.02	0.00	0.04	0.01	0.00	0.00
5	1	6.75	6.74	6.73	6.75	6.73	6.73
	2	6.70	6.72	6.70	6.74	6.74	6.74
	Avg.	6.73	6.73	6.71	6.75	6.73	6.74
	St. dev.	0.03	0.01	0.01	0.01	0.01	0.00
10	1	6.74	6.70	6.74	6.7	6.69	6.68
	2	6.75	6.70	6.70	6.71	6.69	6.70
	Avg.	6.75	6.70	6.72	6.71	6.69	6.69
	St. dev.	0.01	0.00	0.01	0.01	0.01	0.01

Avg.^a Average of two readings.St. dev.^b Standard deviation of two readings.

Table 10-1 pH of the two soils without associated with microorganisms (control)

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.7°C	1.7°C	17.4°C	1.7°C	1.7°C	17.4°C
0	1	6.76	6.76	6.76	6.68	6.68	6.56
	2	6.76	6.76	6.76	6.68	6.68	6.56
	Avg. ^a	6.76	6.76	6.76	6.68	6.68	6.56
	St. dev.	0.01	0.01	0.01	0.01	0.01	0.01
3	1	6.71	6.71	6.68	6.71	6.74	6.72
	2	6.71	6.71	6.70	6.71	6.74	6.72
	Avg.	6.71	6.71	6.70	6.71	6.74	6.72
	St. dev.	0.01	0.01	0.04	0.00	0.00	0.04
6	1	6.71	6.74	6.71	6.74	6.7	6.70
	2	6.71	6.74	6.71	6.67	6.72	6.69
	Avg.	6.71	6.74	6.71	6.71	6.71	6.70
	St. dev.	0.00	0.00	0.00	0.04	0.04	0.04
9	1	6.71	6.71	6.71	6.69	6.69	6.61
	2	6.68	6.72	6.68	6.64	6.70	6.64
	Avg.	6.69	6.72	6.69	6.66	6.70	6.63
	St. dev.	0.00	0.04	0.00	0.01	0.01	0.00
12	1	6.76	6.71	6.76	6.71	6.7	6.72
	2	6.76	6.76	6.76	6.71	6.71	6.72
	Avg.	6.76	6.74	6.76	6.71	6.71	6.72
	St. dev.	0.04	0.01	0.00	0.00	0.00	0.00

^aAvg.^a Average of two readings

St. dev. Standard deviation of two readings

Table 6-6 *all of the data included with Performance Summary*

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.7°C	1.7°C	12.8°C	1.7°C	1.7°C	12.8°C
0	1	6.76	6.76	6.76	6.76	6.76	6.76
	2	6.73	6.73	6.73	6.76	6.76	6.76
	Avg.	6.75	6.75	6.75	6.76	6.76	6.76
	St. dev.	0.01	0.01	0.01	0.00	0.00	0.00
1	1	6.73	6.74	6.74	6.73	6.74	6.74
	2	6.74	6.73	6.73	6.75	6.76	6.76
	Avg.	6.74	6.74	6.74	6.74	6.75	6.75
	St. dev.	0.01	0.01	0.01	0.01	0.01	0.01
3	1	6.73	6.73	6.73	6.73	6.73	6.73
	2	6.73	6.73	6.73	6.73	6.76	6.76
	Avg.	6.73	6.73	6.73	6.73	6.75	6.75
	St. dev.	0.01	0.01	0.01	0.00	0.01	0.01
7	1	6.76	6.67	6.7	6.68	6.71	6.78
	2	6.71	6.72	6.71	6.68	6.71	6.79
	Avg.	6.74	6.70	6.71	6.68	6.71	6.79
	St. dev.	0.03	0.03	0.01	0.01	0.00	0.01
10	1	6.67	6.7	6.67	6.68	6.71	6.74
	2	6.76	6.72	6.67	6.70	6.76	6.80
	Avg.	6.68	6.71	6.67	6.70	6.74	6.77
	St. dev.	0.04	0.00	0.00	0.01	0.01	0.01

Avg. = Average of two readings

St. dev. = Standard deviation of two readings

Table B-8 pH of Salines with associated with *Sarcodes purpurea*

Storage Time (Days)	Sample	pH					
		Experiment 1 (Storage Temperature)			Experiment 2 (Storage Temperature)		
		1.2°C	7.2°C	12.8°C	1.2°C	7.2°C	12.8°C
0	1	6.76	6.76	6.76	6.69	6.69	6.69
	2	6.75	6.75	6.75	6.69	6.69	6.76
	Avg. ^a	6.76	6.76	6.76	6.69	6.69	6.73
	St. dev.	0.01	0.01	0.01	0.01	0.00	0.00
3	1	6.73	6.73	6.74	6.73	6.76	6.73
	2	6.76	6.75	6.75	6.73	6.76	6.73
	Avg.	6.75	6.74	6.75	6.73	6.76	6.73
	St. dev.	0.01	0.00	0.00	0.01	0.00	0.01
6	1	6.71	6.71	6.71	6.76	6.71	6.69
	2	6.71	6.76	6.75	6.73	6.71	6.70
	Avg.	6.71	6.74	6.73	6.75	6.71	6.70
	St. dev.	0.00	0.01	0.01	0.01	0.01	0.01
9	1	6.73	6.73	6.70	6.76	6.76	6.66
	2	6.73	6.73	6.70	6.68	6.70	6.67
	Avg.	6.74	6.73	6.70	6.68	6.73	6.67
	St. dev.	0.01	0.01	0.00	0.01	0.00	0.00
10	1	6.76	6.73	6.70	6.70	6.70	6.71
	2	6.76	6.74	6.71	6.70	6.70	6.66
	Avg.	6.76	6.74	6.71	6.70	6.70	6.70
	St. dev.	0.00	0.01	0.00	0.00	0.00	0.00

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Table B-10 pH of whole milk inoculated with *Pseudomonas fluorescens* or *Bacillus cereus* in accelerated study

Analysis Time (Days)	Sample	pH					
		Experiment 1 (Inoculated <i>Pseudomonas</i>)			Experiment 2 (Inoculated <i>Bacillus cereus</i>)		
		Control	<i>Pseudomonas fluorescens</i>	<i>Bacillus cereus</i> (avg)	Control	<i>Pseudomonas fluorescens</i>	<i>Bacillus cereus</i> (avg)
1	1	6.81	6.60	6.71	6.66	6.71	6.7
	2	6.64	6.41	6.75	6.71	6.64	6.76
	Avg ^a	6.64	6.44	6.73	6.73	6.76	6.73
	St. dev	0.01	0.01	0.02	0.06	0.06	0.03
4	1	6.79	6.71	6.65	6.71	6.61	6.67
	2	6.71	6.62	6.67	6.76	6.61	6.61
	Avg	6.75	6.67	6.66	6.76	6.61	6.64
	St. dev	0.01	0.04	0.01	0.04	0.05	0.01
6	1	6.64	6.61	6.67	6.64	6.66	6.76
	2	6.67	6.61	6.67	6.71	6.66	6.66
	Avg	6.66	6.64	6.67	6.70	6.66	6.76
	St. dev	0.01	0.02	0.00	0.03	0.06	0.01
8	1	6.61	6.67	6.67	6.62	6.79	6.73
	2	6.71	6.69	6.66	6.68	6.76	6.76
	Avg	6.68	6.68	6.67	6.65	6.77	6.75
	St. dev	0.04	0.01	0.01	0.01	0.02	0.01

Avg.^a Average of two readings

St. dev Standard deviation of two readings

Table B-11. pH of induced fat milk inoculated with *Parabacterium ruminantium* or *Bacillus cereus* in inoculated study

Analysis Time (Days)	Sample	pH					
		Experiment 1 (Inoculated Microorganisms)			Experiment 2 (Inoculated Microorganisms)		
		Control ^a	<i>Bacillus cereus</i>	<i>B. cereus</i> + <i>Parabacterium</i>	Control ^a	<i>Bacillus cereus</i>	<i>B. cereus</i> + <i>Parabacterium</i>
1	1	6.70	6.71	6.71	6.70	6.71	6.70
	2	6.71	6.71	6.68	6.70	6.71	6.70
	Avg. ^b	6.71	6.71	6.70	6.71	6.71	6.70
	St. dev.	0.01	0.01	0.02	0.01	0.01	0.02
2	1	6.64	6.71	6.68	6.70	6.67	6.70
	2	6.67	6.71	6.68	6.70	6.67	6.68
	Avg.	6.66	6.71	6.68	6.70	6.67	6.70
	St. dev.	0.02	0.01	0.01	0.01	0.02	0.02
3	1	6.70	6.70	6.67	6.67	6.64	6.70
	2	6.69	6.70	6.68	6.66	6.61	6.70
	Avg.	6.70	6.70	6.68	6.66	6.64	6.70
	St. dev.	0.01	0.00	0.01	0.01	0.03	0.00
4	1	6.67	6.70	6.67	6.71	6.67	6.74
	2	6.54	6.70	6.67	6.71	6.66	6.74
	Avg.	6.61	6.71	6.67	6.72	6.67	6.74
	St. dev.	0.06	0.01	0.01	0.01	0.01	0.00

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Table B-42. pH of 14-day soils inoculated with *Pseudomonas fluorescens* or *Bacillus cereus* in ecological study

Analysis Time (Days)	Sample	pH					
		Experiment 1 (Overland Microorganisms)			Experiment 2 (Buried Microorganisms)		
		Control ^a	<i>P. fluorescens</i>	<i>B. cereus</i>	Control ^a	<i>P. fluorescens</i>	<i>B. cereus</i>
1	1	6.64	6.78	6.48	6.65	6.93	6.51
	2	6.61	6.63	6.47	6.64	6.56	6.57
	Avg. ^b	6.63	6.69	6.48	6.63	6.75	6.53
	St. dev.	0.01	0.04	0.01	0.01	0.03	0.02
4	1	6.58	6.39	6.45	6.71	6.34	6.59
	2	6.53	6.41	6.32	6.69	6.23	6.49
	Avg.	6.56	6.40	6.33	6.71	6.29	6.45
	St. dev.	0.04	0.04	0.04	0.04	0.05	0.04
8	1	6.41	6.17	6.48	6.64	6.28	6.67
	2	6.43	6.11	6.19	6.64	6.27	6.19
	Avg.	6.42	6.15	6.33	6.65	6.28	6.43
	St. dev.	0.01	0.04	0.09	0.01	0.01	0.24
14	1	6.70	6.9	6.70	6.71	6.45	6.71
	2	6.73	6.81	6.70	6.73	6.68	6.71
	Avg.	6.72	6.85	6.70	6.72	6.57	6.71
	St. dev.	0.01	0.01	0.03	0.00	0.03	0.00

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Table 9-4) pH of whole milk inoculated with *Pseudomonas fluorescens* and *Bacillus cereus*

Storage Time (Days)	Sample	pH					
		Control			<i>Pseudomonas fluorescens</i> and <i>Bacillus cereus</i>		
		Storage Temperature			Storage Temperature		
		1°C	7°C	12°C	1°C	7°C	12°C
0	1	6.68	6.68	6.68	6.70	6.71	6.71
	2	6.71	6.71	6.71	6.70	6.69	6.70
	Avg. ^a	6.69	6.69	6.69	6.70	6.70	6.70
	St. dev.	0.02	0.02	0.02	0.02	0.01	0.01
1	1	6.67	6.67	6.67	6.68	6.66	6.71
	2	6.68	6.67	6.67	6.67	6.66	6.70
	Avg.	6.68	6.67	6.67	6.67	6.67	6.70
	St. dev.	0.01	0.00	0.00	0.01	0.01	0.02
2	1	6.69	6.70	6.69	6.68	6.70	6.70
	2	6.69	6.70	6.67	6.68	6.69	6.70
	Avg.	6.69	6.70	6.68	6.68	6.69	6.70
	St. dev.	0.02	0.00	0.01	0.02	0.02	0.02
3	1	6.71	6.71	6.70	6.72	6.70	6.70
	2	6.67	6.70	6.70	6.70	6.71	6.70
	Avg.	6.70	6.71	6.70	6.70	6.70	6.70
	St. dev.	0.01	0.01	0.00	0.00	0.01	0.00
10	1	6.71	6.71	6.71	6.70	6.70	6.70
	2	6.71	6.71	6.71	6.70	6.70	6.70
	Avg.	6.71	6.71	6.71	6.70	6.70	6.70
	St. dev.	0.00	0.00	0.00	0.00	0.00	0.00

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

Table B-14 pH of whole milk, inoculated with *Pseudomonas fluorescens* and *Bacillus cereus* in accelerated study

Analysis Time (Days)	Sample	pH	
		Control	<i>Pseudomonas fluorescens</i> and <i>Bacillus cereus</i>
1	1	6.71	6.18
	2	6.68	6.08
	Avg. ^a	6.70	6.13
	St. dev.	0.02	0.01
4	1	6.62	6.73
	2	6.63	6.61
	Avg.	6.64	6.67
	St. dev.	0.01	0.06
6	1	6.70	6.76
	2	6.70	6.78
	Avg.	6.70	6.77
	St. dev.	0.00	0.04
8	1	6.63	6.68
	2	6.68	6.78
	Avg.	6.66	6.73
	St. dev.	0.03	0.05

Avg.^a Average of two readings

St. dev. Standard deviation of two readings

APPENDIX C DATA FOR SENSORY ANALYSIS

Table C-1 Sensory data of whole milk without inoculated with microorganisms (control)

Storage Temperature	Protein	Sensory Score									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
		0	2	3	5	7	10	0	2	3	5
17°C	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
12°C	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
10°C	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0

Table C-3. Storage data of tubule with insulated with *Permethane* glycerol

Storage Temperature	Phase	Storage Score									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
		0	1	2	3	10	0	1	2	3	10
1 °C	1	8	1	0	0	1	0	1	0	1	2
	2	8	3	1	1	4	0	2	0	3	4
	3	8	0	0	1	1	0	1	1	2	1
	4	8	1	0	1	1	0	1	0	1	1
	5	0	0	4	2	2	0	1	0	0	0
	6	0	1	2	4	4	0	2	1	1	1
	7	0	1	1	2	0	0	1	0	1	1
	8	8	1	1	2	1	0	1	1	1	1
	9	8	3	1	4	1	0	3	1	2	1
	10	8	0	0	1	0	0	0	0	0	1
7 °C	1	8	1	0	1	1	0	0	1	1	2
	2	0	2	0	2	0	0	2	1	1	0
	3	0	1	0	2	1	0	2	2	4	2
	4	0	1	0	1	0	0	2	4	1	4
	5	8	1	0	1	1	0	2	0	2	0
	6	8	1	1	1	0	0	1	0	1	4
	7	8	0	0	1	1	0	0	0	2	1
	8	8	1	4	1	1	0	1	1	2	1
	9	8	1	1	1	1	0	2	2	0	0
	10	8	0	0	1	1	0	1	1	2	1
10 °C	1	0	2	0	1	0	0	0	4	0	0
	2	8	1	1	0	0	0	0	0	0	10
	3	8	1	4	1	1	0	2	1	0	1
	4	0	1	1	4	10	0	1	5	0	0
	5	0	1	0	4	10	0	1	2	5	0
	6	0	0	1	0	10	0	0	1	1	0
	7	0	0	0	1	10	0	2	1	0	0
	8	0	2	0	0	0	0	2	1	2	0
	9	0	1	4	1	10	0	2	0	0	10
	10	0	1	1	1	0	0	0	4	0	0

Table C.3. Recovery time of whole milk inoculated with *Acetivibrio* spp.

Storage Temperature	Position	Recovery Time									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
1 °C	1	0	1	1	2	10	0	1	1	2	10
	2	0	1	0	1	1	0	0	0	1	1
	3	0	1	0	1	1	0	0	1	0	0
	4	0	1	0	0	0	0	0	1	0	1
	5	0	0	0	1	1	0	1	1	0	0
	6	0	0	0	1	0	0	1	0	0	1
	7	0	1	0	1	0	0	0	1	0	1
	8	0	0	0	1	0	0	0	0	1	1
	9	0	0	0	1	0	0	0	0	1	1
	10	0	1	0	0	1	0	1	1	1	1
7.5 °C	1	0	1	0	0	1	0	0	0	1	1
	2	0	1	1	1	0	0	1	0	0	0
	3	0	0	0	1	1	0	0	1	1	0
	4	0	1	1	1	1	0	1	0	0	0
	5	0	1	1	1	0	1	1	1	0	0
	6	0	1	1	1	1	0	1	1	1	0
	7	0	1	0	1	1	0	0	0	0	1
	8	0	1	1	1	1	0	0	1	1	1
	9	0	1	1	0	1	0	1	1	1	0
	10	0	0	0	0	0	0	0	1	0	1
15 °C	1	0	0	0	0	0	1	0	0	0	10
	2	0	1	1	1	1	0	1	0	0	0
	3	0	0	0	1	1	0	1	0	1	0
	4	0	0	1	0	1	0	1	0	0	1
	5	0	0	0	1	0	1	0	1	1	1
	6	0	1	1	1	1	0	1	1	1	1
	7	0	0	1	1	1	0	0	1	0	1
	8	0	0	1	1	1	0	1	1	1	1
	9	0	1	1	1	1	0	1	1	1	1
	10	0	1	1	1	1	0	1	1	1	1

Table C-6 Recovery data of vehicle with fire incident control

Average Time	Pondus	Sampling System									
		Experiment 1					Experiment 2				
		Recovery Time (Days)					Recovery Time (Days)				
		0	1	2	3	4	0	1	2	3	4
1.7%	1	-	-	-	-	-	-	-	1	0	0
	2	-	-	-	-	-	-	-	0	2	1
	3	-	-	-	-	-	1	-	2	1	0
	4	-	-	-	-	-	-	-	1	1	0
	5	1	-	-	-	-	-	-	0	1	0
	6	1	-	-	-	-	-	-	1	1	0
	7	1	-	-	-	-	-	-	0	0	0
	8	-	-	-	-	-	-	-	2	1	1
	9	-	-	-	-	-	1	-	2	0	0
	10	-	-	-	-	-	-	-	0	1	0
7.2%	1	-	0	0	1	0	-	0	1	0	1
	2	-	0	0	0	0	-	0	1	1	0
	3	-	1	1	1	1	-	1	2	0	0
	4	-	0	1	1	0	-	0	0	0	1
	5	-	1	0	1	1	-	0	0	0	0
	6	-	0	0	0	0	1	1	1	1	0
	7	-	0	1	1	0	-	0	0	0	0
	8	-	0	1	1	1	-	1	1	0	1
	9	-	0	1	1	1	-	0	1	0	0
	10	-	0	0	0	1	-	0	1	1	0
13.9%	1	-	0	0	1	1	-	0	0	0	1
	2	-	0	1	0	0	-	0	1	0	0
	3	-	1	0	1	1	-	1	2	1	1
	4	1	0	0	0	0	-	0	1	1	0
	5	-	1	0	0	1	-	0	0	0	0
	6	-	0	0	0	1	-	0	1	1	0
	7	-	0	0	1	1	-	0	1	1	0
	8	-	0	1	0	1	-	2	0	0	1
	9	-	0	1	0	1	-	0	1	0	0
	10	-	0	0	1	1	-	0	1	0	0

Table C-1 Shelf-life of reduced-fat milk (without inoculated) with microorganisms (control)

Storage Temperature	Pondok	Sensory Score									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
17°C	1	0	1	1	2	10	0	1	2	7	10
	2	0	0	0	0	1	1	0	0	0	0
	3	0	0	0	0	0	0	0	1	0	0
	4	0	0	0	0	1	1	0	0	0	0
	5	0	0	0	1	1	0	0	1	0	1
	6	0	0	1	1	4	1	0	1	1	0
	7	0	0	1	1	1	1	0	0	0	1
	8	0	0	0	4	2	1	0	1	0	1
	9	0	0	1	0	0	1	0	1	0	0
	10	0	0	1	0	1	0	0	0	1	0
13°C	1	0	0	0	0	0	0	0	1	0	0
	2	0	0	1	1	1	1	0	0	0	1
	3	0	0	1	2	2	1	0	4	1	0
	4	0	0	0	3	2	0	0	3	0	0
	5	0	0	0	1	1	1	0	0	0	0
	6	0	0	1	1	2	0	0	1	1	0
	7	0	0	1	0	0	0	0	0	0	1
	8	0	0	0	1	2	1	0	4	0	0
	9	0	0	0	1	1	1	0	2	1	0
	10	0	0	1	0	0	1	0	1	0	0
10°C	1	0	0	0	0	0	1	0	0	0	0
	2	0	1	1	0	2	0	0	1	0	0
	3	0	1	0	2	4	0	0	2	0	1
	4	0	0	0	4	1	0	1	0	0	1
	5	0	0	1	4	1	0	0	0	1	1
	6	0	1	1	4	1	0	0	1	1	1
	7	0	0	0	0	2	1	0	2	1	0
	8	0	1	0	1	1	0	0	0	0	0
	9	0	4	0	1	2	0	1	0	1	0
	10	0	0	0	1	0	1	0	0	0	1

Table C-6. Recovery time of reduced-fat milk inoculated with *Pseudomonas fluorescens*

Storage Temperature	Inoculated	Sensory Scores									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
17°C	1	3	3	3	3	18	3	3	3	3	18
	2	3	3	3	3	4	3	3	3	3	3
	3	3	3	3	4	3	3	4	3	3	3
	4	3	3	3	3	3	3	3	3	3	3
	5	3	3	3	3	3	3	3	3	3	3
	6	3	3	4	3	3	3	3	4	3	3
	7	3	3	3	3	3	3	3	3	3	3
	8	3	3	3	4	3	3	3	3	3	3
	9	3	3	3	3	4	3	3	3	3	3
	10	3	3	3	3	4	3	3	3	3	3
13°C	1	3	3	3	3	4	3	3	3	3	3
	2	3	3	3	3	3	3	3	4	4	3
	3	3	3	3	3	3	3	3	3	4	3
	4	3	3	4	4	3	4	3	3	3	3
	5	3	3	3	3	4	4	3	3	4	3
	6	3	3	4	3	4	3	3	3	3	18
	7	3	3	3	3	4	3	3	3	4	3
	8	3	3	3	3	3	3	3	3	3	3
	9	3	3	3	4	3	3	3	3	4	3
	10	3	3	3	3	3	3	3	3	3	3
10.8°C	1	3	3	3	3	3	3	3	3	3	3
	2	3	3	4	3	3	10	3	3	3	3
	3	3	3	3	4	3	3	3	3	10	3
	4	3	3	10	3	10	3	3	3	3	10
	5	3	3	4	3	3	3	3	3	3	10
	6	3	3	3	3	3	10	3	3	3	10
	7	3	3	3	10	10	3	3	3	3	10
	8	3	3	3	3	3	3	3	3	3	3
	9	3	3	3	4	3	10	3	3	3	3
	10	3	3	3	3	3	10	3	3	3	3

Table G-8 Recovery data of rainbow trout for brook trout control

Storage Temperature	Pond	Recovery System									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
		0	1	2	7	10	0	1	2	7	10
17°C	1	-	-	-	-	-	-	0	0	0	0
	2	-	-	-	-	-	-	1	0	1	0
	3	-	-	-	-	-	-	1	1	1	0
	4	-	1	1	-	-	-	2	0	1	0
	5	-	1	-	-	-	-	0	0	2	1
	6	-	-	-	-	-	-	0	0	0	0
	7	-	-	-	-	-	-	0	0	1	1
	8	-	-	-	-	-	-	0	0	2	1
	9	-	-	-	-	-	-	1	0	1	1
	10	-	-	-	-	-	-	1	0	4	1
12°C	1	-	2	0	1	1	-	0	1	0	0
	2	-	4	1	0	0	-	0	0	1	0
	3	-	0	1	2	1	-	2	4	1	1
	4	-	0	1	1	1	-	2	1	1	1
	5	-	1	1	0	0	-	0	0	1	0
	6	-	0	1	0	0	-	1	0	0	0
	7	-	0	1	1	1	-	0	0	0	0
	8	-	0	1	1	1	-	1	1	0	1
	9	-	0	1	0	1	-	1	0	0	0
	10	-	0	0	0	1	-	0	1	1	0
10-14°C	1	-	0	0	1	1	-	0	0	0	0
	2	-	0	1	1	1	-	0	0	0	0
	3	-	0	1	2	2	-	0	1	1	1
	4	-	0	2	0	1	-	0	1	0	0
	5	-	0	1	0	0	-	0	1	1	1
	6	-	0	0	0	0	-	0	0	2	1
	7	-	0	0	0	0	-	0	0	2	1
	8	-	0	1	0	1	-	1	1	0	1
	9	-	1	1	0	0	-	0	0	0	0
	10	-	0	1	1	0	-	0	0	1	0

Table C-9 Recovery data of Ba-free milk tablets insulated with microcapsules (control)

Storage Temperature	Parameter	Recovery System									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
17°C	1	0	0	3	3	10	0	1	5	7	10
	2	0	0	0	1	3	0	0	0	1	0
	3	0	0	0	4	0	2	0	2	3	0
	4	0	0	0	0	0	0	1	0	3	1
	5	0	0	0	1	0	4	1	1	1	0
	6	0	0	4	1	1	0	0	0	0	1
	7	0	0	3	0	0	0	0	0	0	0
	8	0	0	0	1	2	0	0	1	1	0
	9	0	0	0	2	1	1	0	0	1	0
	10	0	0	0	0	0	1	0	0	0	0
7°C	1	0	0	0	1	1	0	1	1	0	1
	2	0	1	1	1	1	0	1	1	1	0
	3	0	0	1	1	1	0	4	2	2	1
	4	0	0	1	1	1	0	2	2	1	1
	5	0	1	0	4	1	0	0	0	1	0
	6	0	1	1	1	1	0	0	1	1	1
	7	0	1	1	1	1	0	0	0	0	0
	8	0	1	1	2	1	0	1	1	1	1
	9	0	1	1	1	1	0	1	0	1	0
	10	0	0	1	1	1	0	0	0	0	0
12.5°C	1	0	0	1	0	0	0	0	0	0	0
	2	0	0	1	1	4	0	1	1	0	0
	3	0	0	0	1	4	0	2	1	1	1
	4	0	1	1	4	1	0	1	0	0	0
	5	0	0	1	1	1	0	0	0	0	0
	6	0	1	1	4	2	0	0	1	1	1
	7	0	1	1	2	0	0	0	0	0	1
	8	0	0	1	2	1	0	1	0	1	1
	9	0	0	0	1	2	0	1	0	0	1
	10	0	0	0	1	0	0	0	0	1	1

Table C-10 Storage data of facilities with associated with Phenol-based Acrylates

Storage Temperature	Facilities	Storage Cycles									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
		0	1	2	3	4	5	6	7	8	9
17°C	1	0	0	0	1	1	2	0	2	1	1
	2	0	0	0	2	1	0	0	0	2	4
	3	0	0	1	1	2	4	0	2	2	2
	4	0	1	0	0	1	0	0	0	0	4
	5	0	1	1	1	1	0	0	2	0	1
	6	0	1	1	1	1	0	0	0	1	1
	7	0	1	1	1	2	1	0	2	0	1
	8	0	0	0	2	1	2	0	0	0	1
	9	0	0	1	1	2	0	0	1	2	4
	10	0	0	0	2	2	0	0	1	2	1
17°C	1	0	0	1	1	4	0	0	1	0	1
	2	0	2	1	4	0	0	0	4	4	1
	3	0	0	1	2	1	0	1	2	2	0
	4	0	0	1	1	1	2	0	0	0	1
	5	0	1	1	4	2	0	2	0	0	0
	6	0	0	1	4	0	0	1	2	2	0
	7	0	0	0	2	1	2	0	2	0	1
	8	0	1	1	1	4	0	0	2	0	0
	9	0	0	2	2	4	0	0	1	0	0
	10	0	0	0	1	2	0	0	1	2	1
18°C	1	0	2	0	0	10	10	0	1	0	0
	2	0	2	0	0	7	10	0	4	7	10
	3	0	2	0	0	0	10	0	0	0	10
	4	0	1	1	0	0	10	0	0	0	10
	5	0	0	0	1	0	10	0	4	5	10
	6	0	2	0	0	0	10	0	1	0	10
	7	0	2	0	0	1	10	0	0	0	10
	8	0	1	1	1	0	0	0	0	0	10
	9	0	1	1	2	1	10	0	1	0	10
	10	0	0	0	2	10	10	0	2	0	0

Table C-11 Accuracy data of *Isotest* with associated with the file computer

Storage Temperature (Fahrenheit)	Storage Time (Days)	Storage Range									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
		0	1	2	3	4	5	6	7	8	9
17°C	1	0	0	0	0	1	0	0	1	2	4
	2	0	1	2	2	4	0	1	4	2	1
	3	0	0	1	2	4	0	0	1	3	1
	4	0	1	0	1	0	0	1	1	0	1
	5	0	1	2	2	1	0	1	0	0	0
	6	0	1	1	2	4	0	1	1	2	2
	7	0	2	1	2	0	0	1	0	1	1
	8	0	0	0	2	1	0	1	1	1	1
	9	0	2	1	2	0	0	0	1	2	2
	10	0	0	1	1	4	0	1	2	2	2
17°C	1	0	1	1	1	2	0	2	4	2	0
	2	0	0	1	2	1	0	2	0	0	1
	3	0	1	1	2	4	0	1	1	1	1
	4	0	2	2	1	0	0	2	1	1	1
	5	0	2	1	1	2	0	1	0	0	1
	6	0	2	1	2	4	0	1	2	1	2
	7	0	0	1	2	0	0	1	2	2	2
	8	0	0	1	2	1	0	2	2	1	1
	9	0	0	1	2	1	0	1	1	1	2
	10	0	0	1	1	4	0	1	2	1	2
18°C	1	0	0	2	4	0	0	2	4	1	2
	2	0	1	1	4	10	0	2	2	1	4
	3	0	0	2	1	4	0	0	2	4	4
	4	0	1	2	1	0	0	2	1	2	2
	5	0	1	2	1	10	0	2	2	2	1
	6	0	1	1	2	1	0	1	1	4	2
	7	0	1	2	0	0	0	1	1	4	4
	8	0	2	2	1	1	0	2	1	1	1
	9	0	1	1	2	0	0	0	1	2	2
	10	0	0	1	1	1	0	2	0	0	2

Table C-11: Storage data of the data with the battery control

Storage Temperature	Position	Battery Voltage									
		Experiment 1					Experiment 2				
		Storage Time (Days)					Storage Time (Days)				
		0	1	2	3	4	0	1	2	3	4
17°C	1	-	1	0	0	0	-	1	0	0	1
	2	-	1	0	0	0	-	0	0	0	1
	3	-	1	0	0	0	-	0	1	0	1
	4	-	2	2	0	1	-	0	0	0	0
	5	-	0	0	2	2	-	0	0	0	1
	6	-	0	0	0	0	-	0	0	0	0
	7	-	1	0	0	1	-	0	1	0	0
	8	-	1	0	0	1	-	0	2	0	0
	9	-	1	0	0	0	-	0	0	1	0
	10	-	1	1	0	0	-	0	0	0	0
17°C	1	-	1	0	0	0	-	0	0	0	0
	2	-	0	0	0	0	-	0	0	0	0
	3	-	1	0	0	0	-	2	1	1	1
	4	-	1	0	0	0	-	0	0	0	0
	5	-	0	0	1	1	-	0	1	0	0
	6	-	0	0	0	0	-	0	0	0	0
	7	-	0	0	0	0	-	0	0	0	0
	8	-	1	0	0	2	-	0	0	0	0
	9	-	0	0	0	0	-	0	0	0	0
	10	-	0	0	0	0	-	0	0	0	0
18.5°C	1	-	0	0	0	0	-	0	0	0	0
	2	-	0	0	0	1	-	0	0	0	0
	3	-	0	0	0	0	-	2	1	2	1
	4	-	0	0	0	0	-	0	0	0	0
	5	-	0	0	1	1	-	0	0	1	0
	6	-	0	0	0	0	-	0	0	0	0
	7	-	0	0	0	0	-	0	0	1	0
	8	-	2	0	1	1	-	0	1	0	0
	9	-	0	0	0	0	-	0	0	0	1
	10	-	1	0	0	0	-	0	0	0	0

Table C-11 Storage data of whole milk, sterilized milk, *Pseudomonas fluorescens* and *Bacillus coagulans*

Storage Temperature	Profile	Storage Status									
		Control					<i>Pseudomonas fluorescens</i> and <i>Bacillus coagulans</i>				
		Storage Time (Days)					Storage Time (Days)				
		0	1	2	3	10	0	1	2	3	10
17°C	1	0	1	1	2	0	0	0	0	1	1
	2	0	0	1	2	1	0	1	1	0	1
	3	0	0	1	2	0	0	0	2	2	4
	4	0	0	1	2	0	0	1	0	0	1
	5	0	1	1	0	1	0	0	0	0	1
	6	0	0	0	0	0	1	0	1	1	0
	7	0	1	1	1	0	0	1	0	2	3
	8	0	0	1	2	1	0	1	1	1	1
	9	0	0	1	0	0	0	1	0	1	1
	10	0	0	1	1	0	0	1	2	1	1
7.2°C	1	0	1	0	0	0	0	0	1	1	0
	2	0	0	0	0	1	0	0	1	1	4
	3	0	0	1	2	0	0	1	1	2	1
	4	0	0	1	1	1	0	1	1	0	1
	5	0	0	1	2	0	0	0	0	0	0
	6	0	0	1	1	1	0	1	2	2	0
	7	0	0	0	0	1	0	1	1	4	1
	8	0	0	1	1	1	0	1	2	4	0
	9	0	0	1	0	0	0	1	1	1	1
	10	0	0	1	2	1	0	1	2	4	1
12.8°C	1	0	0	0	0	0	0	0	0	10	10
	2	0	0	0	0	2	0	1	0	0	10
	3	0	0	1	1	1	0	1	0	7	10
	4	0	0	0	0	0	0	1	1	0	10
	5	0	0	2	0	1	0	0	0	0	10
	6	0	0	0	1	1	0	2	2	7	10
	7	0	0	0	2	1	0	1	1	10	10
	8	0	0	1	1	1	0	1	2	0	10
	9	0	0	1	1	1	0	1	2	3	10
	10	0	0	2	1	0	0	1	0	0	10

Table C-14 Sensory data of whole milk inoculated with *Pseudomonas fluorescens* and bacterial compliance for holding control

Storage Temperature	Passive	Sensory Scores				
		Storage Time (Days)				
		0	1	2	3	4
1 °C	1	0	0	0	1	1
	2	0	0	0	0	0
	3	0	0	1	4	2
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
	7	0	0	0	1	1
	8	0	0	1		0
	9	0	0	0	0	0
	10	0	0	1	0	0
7 °C	1	0	0	0	0	0
	2	0	0	0	0	1
	3	0	1		1	0
	4	0	0	1	0	0
	5	0	0	0	1	1
	6	0	0	0	0	0
	7	0	0	0	1	0
	8	0	0	1	1	0
	9	0	0	0	0	0
	10	0	0	1	1	0
12 °C	1	0	1	0	2	0
	2	0	0	0	0	0
	3	0	1	1	1	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
	7	0	0	0	1	0
	8	0	0	1	1	1
	9	0	0	0	0	0
	10	0	0	1	0	0

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BIOGRAPHICAL SKETCH

Figen Koral works in Izmir, Turkey. She attended the American College Institute in Izmir, where she became interested in food sciences. Then she studied in the Food Engineering Department of Ege University, Izmir, where she earned her bachelor of science degree in food engineering in August 1992. After graduation, she started her master's studies in the same department.

In June 1993, she received a scholarship from Celal Bayar University through the Higher Education Institute of Turkey to conduct her graduate studies abroad. In May 1996, she received her master of science degree in animal and food industries from Clemson University. She was listed in the "Profiles of Excellence" in 1995 and 1996 and became a member of the Agricultural Honor Society, Clemson Sigma Delta.

In August 1996, she started her Ph.D. program in the Food Science and Human Nutrition Department of the University of Florida. Figen expects to receive her Doctor of philosophy degree in food science and human nutrition in May 2000. She plans to return to Turkey to become a faculty member at Celal Bayar University in Manisa.

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David G. Bauman, Chair
Professor of Food Science and
Human Nutrition

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Gary H. Roffeck
Professor of Food Science
and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy



Charles A. Sims
Professor of Food Science and
Human Nutrition

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Sally K. Williams
Associate Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy



Kenneth M. Foster
Associate Professor of Statistics

